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A database development of gas chromatographic metabolites identified in Nilavembu kudineer extracted in different solvents and comparison with previous data analysis.

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Abstract

Dengue, one of the major threats to the human population that can be controlled by preventive measures and hygienic-sanitary practices. The currently available Siddha formulation for dengue is Nilavembu Kudineer, which has nine plant components composed of bioactive compounds which are actively participating in increasing the platelet count in dengue-affected patients. In this study, screened the various compounds obtained from the Nilavembu kudineer in six different solvents and compared with previous study findings for compounds and a database developed for the easy access of details of the compounds for future aspects. By this, we identified nearly 550 compounds from previous study data and 120 compounds from GC-MS analyzed sample extracted from water, ethanol, methanol, ethyl acetate, acetone and hexane solvent. Among those, 258 compounds were found to be less toxic using OSIRIS and ADMET profiling based on drug score and drug-likeness. From this result, we identified the best extraction method suitable for extracting most of the less-toxic bioactive compounds from different solvents. Based on the this study a database <http://nilavembukudineer.in/> have been developed for user friendly access of details of the compounds and can be further used in drug design and developmental studies.

Keywords: NVK - Nilavembu kudineer, Dengue, OSIRIS, Swiss-ADME.

Introduction

Female *Aedes aegypti* and *Aedes albopictus* mosquitoes spread dengue fever, an arthropod-borne disease through biting and injection of virus into blood stream of host. Dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome are all caused by the dengue virus (DSS). Capillary leakage, thrombocytopenia, altered haemostasias, and liver damage are all symptoms of DHF and DSS, as evidenced by a rise in aspartate aminotransferase and alanine aminotransferase¹. Dengue fever is transmitted seasonally, thus it is most common during the rainy season. The mosquito extrinsic incubation period, biting frequency, and vector life span are all favoured by this temperature. The propagation of dengue fever is impacted by urban poverty and a lack of sanitary sanitation. The available dengue vaccine Dengvaxia is a chimera of structural genes of four DENVs with non-structural yellow fever vaccine genome (Halstead, S. B. 2008). The vaccine efficacy and safety in placebo-controlled clinical trials was conducted among 35,000 children of age group 2-16 years in ten endemic countries. Efficacy results were not appropriate. As the serostatus was 14.4% in 8-9 years the vaccine was not recommended by the committee for children below 9 years (Anderson et.al., 1997). Another vaccine, TAK 003, a live attenuated vaccine containing DENV-2 strain and chimeric strains of DENV-1, DENV-3, DENV-4 is in clinical trial. The another tetravalent live-attenuated vaccine (LATV) is also in trial which consists of mutated DENV-1,3,4 and chimera of DENV-2. This vaccine is administered as a single dose only in Brazil (Halstead, 2019). Hydroxychloroquine and chloroquine are being used as antivirals to treat a variety of viral infections. Based on the single well-designed RCT values, sample size, and bias of low risks, high certainty evidence also indicated these medications cannot be used for dengue (Rodrigo, 2020). Currently there is no vaccine or drug available for dengue. In Siddha, traditional method by which polyherbal formulation is used as a treatment for all diseases. The physiological system, according to Siddha, is primarily made up of Vatham, Pitham, and Karpam, collectively known as Tridhosham. Siddha medicine has a lot of benefits (Jain et.al., 2020). Medicinal plants are classical in treatment and recovery of many infectious diseases as they have numerous healing properties. The studies also proved that usage of medicinal plants as drugs for treatment will not end in any side effects. By this nilavembu kudineer was proved as best curative for Dengue and Chikungunya in hospitalized

affected patients (Koushik, 2015). Nilavamebu kudineer is prescribed as efficient therapy for thrombocytopenia caused in dengue affected patients. Though the concoction of 9 plants of equal quantity in nilavembu kudineer is proved to be efficient during dengue infection for boosting immune system the efficacy of nilavembu kudineer by antiviral activity is yet to be studied. The nilavembu kudineer is the concoction of plants *Andrographis Paniculata* (Nilavembu), *Plectranthus Vettiveroides* (Vilamichai Ver), *Vetiveria zizanioides* (Vetiver), *Zingiber Officinale* (Chukku), *Piper Nigrum* (Milagu), *Cyperus Rotundus* (Korai Kizhangu), *Santalum Album* (Santanam), *Trichosanthes Cucumerina* (Peyputtal), *Mollugo Cerviana* (Parpadagam). All these plant extracts and also individual components are used widely for the treatment of fever, inflammation, arthralgia, arthritis, gastric ulcer, jaundice and other debility conditions. Nilavembu kudineer chooranam is proved to possess the better therapeutic action than other NSAIDs and analgesic (Anbarasu et.al., 2011). *Piper Nigrum* (Milagu) cultivated worldwide and consumed as fresh, dry or powdered spices. Commonly called pepper is grown in tropical regions of Brazil, Indonesia and India. Pepper is majorly used as a stimulant, carminative, diuretic, anticholera, anti-asthmatic, analgesic, anti-inflammatory, hepatoprotective etc. the commonly found alkaloid Piperine shown to possess enormous pharmacological properties. *Andrographis Paniculata* (Nilavembu) is the traditional herb which helps as analgesic, anti-inflammatory and also helps in many respiratory problems (Zahin et.al., 2021). Hence the need of identifying bioactive metabolites for dengue using plant products are in need and this database will provide the researchers to easily identify the specific less toxic compounds for further research in antiviral studies.

Materials and Methods

Sample collection and extraction

The components of nilavembu kudineer *Andrographis Paniculata* (Nilavembu), *Plectranthus Vettiveroides* (Vilamichai Ver), *Vetiveria zizanioides* (Vetiver), *Zingiber Officinale* (Chukku), *Piper Nigrum* (Milagu), *Cyperus Rotundus* (Korai Kizhangu), *Santalum Album* (Santanam), *Trichosanthes Cucumerina* (Peyputtal), *Mollugo Cerviana* (Parpadagam) were freshly collected from IMCOPs (The Indian Medical Practitioners Co-operative Pharmacy and Stores Ltd.), dried and powdered using motor and pestle. Each individual powdered components was added in equal amount to the solvents (ethanol, methanol, ethyl acetate, hexane, acetone and aqueous) and soaked for 10 days. After 10 days the mixture was filtered and dried and compounds were obtained. The aqueous extract was obtained by boiling the

equal amount of sample in water bath for 55°C for 30 mins. After boiling the mixture was filtered and concentrated using rotary evaporator and lyophilized.

GC-MS analysis

The crude extracts were lyophilized and then analyzed using Agilent 7890B GC using HP_5MS 5% Phenyl Methyl Silox column of 30m X 250µm X 0.25µm at -60°C to 325°C. The mass spectrum of compounds present in the extracts were by obtained by electron ionization at 70eV and the detector operates in scan mode 30-500 Da atomic units. The peak table with area and percentage was compared with NIST MS2011 library and identified few compounds.

Drug-Likeness by Insilco analysis

The GC-MS analyzed 120 compounds from six different extraction of Nilavembu kudineer were checked for toxicity and drug likeness using OSIRIS and SWISS-ADMET profiling. The GC-MS analyzed compounds were identified using NCBI-PubChem and molecular weight, molecular structure and canonical SMILES results were identified. Based on the canonical SMILES that were entered in OSIRIS the toxicity of the compounds were identified. Then the results obtained from OSIRIS and SWISS-ADMET Insilco pharmacokinetics the compounds were analyzed. The results were interpreted based on Lipinski rules and drug score which determines the different levels and organ toxicities were used to predict the low, medium and high toxicity of compounds for drug selection.

Results

GC-MS analysis of compounds in ethanol, methanol, ethyl acetate, hexane, acetone and aqueous

Gas chromatographic analysis of the Nilavembu Kudineer powder extracts showed 23, 36, 48, 47, 41, 54 bioactive compounds in ethanol, methanol, ethyl acetate, hexane, acetone and aqueous extracts respectively.

Drug-Likeness by In-silco analysis

When the canonical SMILES of 120 compounds identified in GC and 550 reviewed from past data analysis using SWISS-ADME toxicity profiling 258 compounds were selected based under Lipinski rule 5 while eliminating compounds which were not under the rule. The compounds which fall in Lipinski rule of 5 have satisfied rules of less than 500 molecular weight, logP value less than 5 which denotes lipophilicity, less than 5 hydrogen donors and less than 10 hydrogen acceptors. And also, the physicochemical, solubility and pharmacokinetics properties of compounds were identified.

Database development

A database (<http://nilavembukudineer.in/>) has been developed and inputs of the compounds identified were added in the database for the easy accessibility for researchers and students. This database will provide details of the compounds like molecular weight and formula, extraction method and screening method (GC-MS, HPLC, NMR) used, drug-likeness and drug score, pubchem ID if previous studies were performed.

Conclusion

Hence, in recent years studies on traditional medicines are booming. The *in-silico* studies providing the resourceful data for the *in-vitro* and *in-vivo* studies. Finding rapid and efficient treatment solutions to combat dengue and SARS-CoV has become a significant global concern. Because the process of developing new therapeutic vaccinations and synthetic drugs takes several years, it is necessary to adapt alternative treatments or medications to combat deadly viral infections. Natural product-based alternative therapy appears to be a valuable source for rapid development of antiviral medications. Considering the huge public health impact of Dengue and other viral infections in India, information from the present study may be utilized in devising an effective disease control strategies by using NVK in prophylaxis during outbreaks. Since ancient times, traditional medicines have been used as immune system boosters and as a promising treatment for a wide range of illnesses. This study examined the phytochemical components in Nilavembu kudineer and curated the data for the pharmacological applications. Hence, this database provides phyto constituents of medicinal plants and its properties against various diseases. It will be beneficial to have access to the most recent information in the future because this database will be updated depending on further investigations. Overall, this investigation has identified a putative phytochemicals with potential inhibitory effects against viruses, which may be used for further investigation, formulation, and drug development.

Discussion

Throughout history, medicinal plants have been utilized extensively to treat a wide range of infectious and noninfectious diseases, particularly in developing nations. Even in wealthy nations, the use of natural therapeutic products derived from plants is growing in popularity (Hunsperger et.al., 2009). According to the World Health Organization (WHO) report, 80% of the world's population gets their medical care from phyto-medicinal sources. This is a result of the widespread perception that plants and their metabolomic products are safe, easily available, and low in adverse effects. Many chemical entities found in medicinal plants have a wide range of biological properties, including the ability to inhibit certain viruses from replicating. Now currently there is no approved and validated drug for targeting Dengue virus. Many traditional medical systems have reported using different herbs to treat dengue fever. Based on their interpretation of the data, traditional herbs like *Euphorbia hirta* and *Carica papaya* have been used for generations in various nations (Guzman et.al., 2016). However there is a lack of proof of concept and understanding of the mechanism of action. In order for an agent to be utilized as a therapeutic product of any value today, it must first be thoroughly evaluated for toxicity and efficacy, and its dosage, toxicity, and mechanism of action must be determined through the use of contemporary laboratory techniques and proper documentation (Altamish et.al., 2022). Important medical bioactive components are shown by GCMS analysis of the NKC ethyl acetate extract (Kamalarajan et.al., 2022). The *in-silico* studies on the potential binding affinity and inhibition of dengue virus structural and non structural proteins by the medicinal plant compounds have been conducted. Validation of these drugs initially evaluated in *in-vitro* and then *in-vivo* is strongly advised. Methanolic extract of *Andrographis paniculata* was able to preserve most of the normal cell morphologies in a study conducted for the preliminary screening investigation for anti-Dengue drug without having a significant cytotoxic effect on the DENV-1infected cells (Mattummal et.al., 2018). Many studies provides an overview of the idea that the compounds found in Nilavembu kudineer choornam (NKC) are frequently utilized as a potent anti-inflammatory that can be used to treat a variety of diseases, including cancer, neurological disorders, aging, and inflammation (Mekala & Murthy, 2020).

Reference:

1. World Health Organization. (1997). *Dengue haemorrhagic fever: diagnosis, treatment, prevention and control*. World Health Organization.

2. Halstead, S. B. (2008). Dengue virus–mosquito interactions. *Annu. Rev. Entomol.*, 53, 273-291.
3. Anderson, R., Wang, S., Osiowy, C., & Issekutz, A. C. (1997). Activation of endothelial cells via antibody-enhanced dengue virus infection of peripheral blood monocytes. *Journal of virology*, 71(6), 4226-4232.
4. Halstead, S. (2019). Recent advances in understanding dengue. *F1000Research*, 8.
5. Rodrigo, C., Fernando, S. D., & Rajapakse, S. (2020). Clinical evidence for repurposing chloroquine and hydroxychloroquine as antiviral agents: a systematic review. *Clinical Microbiology and Infection*, 26(8), 979-987.
6. Jain J, Kumar A, Narayanan V, Ramaswamy RS, Sathiyarajeswaran P, Devi MS, Kannan M, Sunil S. Antiviral activity of ethanolic extract of Nilavembu Kudineer against dengue and chikungunya virus through in vitro evaluation. *Journal of Ayurveda and integrative medicine*. 2020 Jul 1;11(3):329-35.
7. Koushik Kumar, G., Prasanna, G., Marimuthu, T., & Saraswathi, N. T. (2015). Structural basis for complementary and alternative medicine: Phytochemical interaction with non-structural protein 2 protease-a reverse engineering strategy. *Chinese journal of integrative medicine*, 21, 445-452.
8. Anbarasu, K., Manisenthil, K. K., & Ramachandran, S. (2011). Antipyretic, anti-inflammatory and analgesic properties of nilavembu kudineer choornam: a classical preparation used in the treatment of chikungunya fever. *Asian Pacific journal of tropical medicine*, 4(10), 819-823.
9. Zahin, M., Bokhari, N. A., Ahmad, I., Husain, F. M., Althubiani, A. S., Alruways, M. W., ... & Shalawi, M. (2021). Antioxidant, antibacterial, and antimutagenic activity of *Piper nigrum* seeds extracts. *Saudi Journal of Biological Sciences*, 28(9), 5094-5105.
10. Hunsperger, E. A., Yoksan, S., Buchy, P., Nguyen, V. C., Sekaran, S. D., Enria, D. A., ... & Peeling, R. W. (2009). Evaluation of commercially available anti–dengue virus immunoglobulin M tests. *Emerging infectious diseases*, 15(3), 436.
11. De Guzman, G. Q., Dacanay, A. T. L., Andaya, B. A., & Alejandro, G. J. D. (2016). Ethnopharmacological studies on the uses of *Euphorbia hirta* in the treatment of dengue in selected indigenous communities in Pangasinan (Philippines). *Journal of intercultural ethnopharmacology*, 5(3), 239.
12. Altamish, M., Khan, M., Baig, M. S., Pathak, B., Rani, V., Akhtar, J., ... & Krishnan, A. (2022). Therapeutic potential of medicinal plants against dengue infection: A mechanistic viewpoint. *ACS omega*, 7(28), 24048-24065.

13. Kamalarajan, P., Muthuraman, S., Ganesh, M. R., & Valan, M. F. (2020). Phytochemical investigation of nilavembu kudineer chooranam ethyl acetate extract and its ability to reduce intracellular antioxidant levels in THP-I cells. *European Journal of Medicinal Plants*, 30(4), 1-13.
14. Mattummal, R., Gopi, D. K., Parameswaran, S. R., & Narayana, S. K. K. (2018). Bioactive molecules in siddha polyherbal nilavembu kudineer alleviating symptoms of dengue/chikugunya. *Traditional Medicine Research*, 3(5), 215.
15. Mekala, P., & Murthy, T. G. K. (2020). Phytochemical screening and pharmacological update on Kabasura Kudineer choornam and Nilavembu Kudineer Choornam. *Journal of Pharmacognosy and Phytochemistry*, 9(3), 1031-1036.

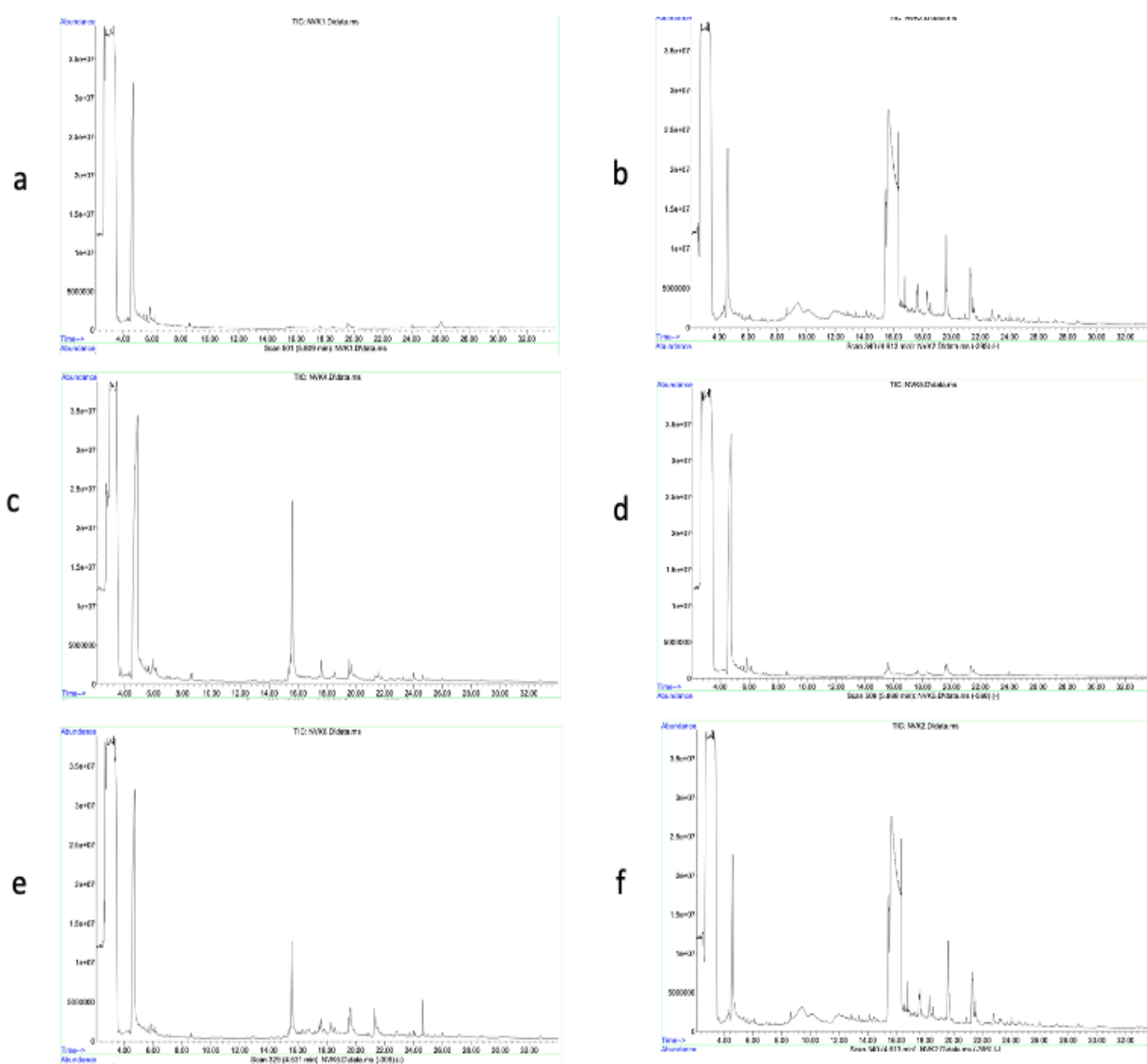


Figure 1: Graphical representation of GC-MS analysis and no. of NVK compounds extracted in (a)Ethanol-23 compounds, (b)Methanol-36 compounds, (c)Ethyl acetate-

48 compounds, (d)Hexane-47 compounds, (e)Acetone-41 compounds, (f)Aqueous-54 compounds.