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GC-MS, FT-IR Analysis and Anti Bacterial Study of Bioactive Compounds of Chundaivattral Chooranam - A Siddha Poly Herbal Formulation

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Abstract: Natural product or natural product structures continued to play a highly significant role in the drug discovery and development process. Chundaivattral Chooranam (CVC) is a Siddha Polyherbal formulation. It consists of equal quantity of dried *Solanum torvum, Murraya koenigii, Mangiferaindica, Carunrox burghianum, Phyllanthus embilica, Punica granatum, Trigonella foenumgraecum.* In the siddha system of medicine, CVC has been used in non-specific diarrhoea. In the present study,the anti-bacterial study was conducted according to the agar diffusion method. Chloramphenicol was used as standard drug. The biological activities of CVC was evaluated for antibacterial properties againt pathogens. The chemical constitutes and the qualitative analysis of CVC was carried out using GC-MS and FTIR analysis for the identification of bioactive components. Results. Were expressed as mean value ± standard error of the mean (SEM). It was observed that Chundai vattral Chooranam herbal formulation (1mg/mL) exerted effective anti-bacterial activity against *Salmonella typhi* (20 ±0.05 mm) and *Shigella flexneri* (21± 0.06 mm) when compared with other bacterial strains respectively. Two major compounds have been identified and the major chemical constituents were 2H-1-benzopyran-6-OL,3,4-dihydro-2,5,7,8-tetramethyl-2-(4-8-12-trimethyl tetradecyl)-2H- -6-yl (MW-410) (b) 1 2 benzenedicarboxylic acid,Mono(2-Ethyl hexyl)Ester (Mw-278). The results of A FTIR analysis confirmed the presence of amide, alkynes, alkanes, carboxylic acids, alkenes, aromatics, aliphatic amines and alkyl halides compounds which showed major. The result of GC-MS and FTIR analysis of CVC revealed the existence of few compounds with potent biological activity, but tended to be present in the formulation even at low concentration levels.
Key words: ChundaivattralChooranam, GC-MS, anti-bacterial activity,FTIR.

1. Introduction

Medicinal plants are the richest bio resource of drugs for traditional systems of medicine. Development of new drugs, especially in area of infectious diseases, represents today one of the most important research. . The problems associated with hospital infections caused by drug resistant bacteria become increasingly evident which is associated with serious gram positive bacterial infections [1]. The use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases [4].It has been found literally thousands of phytochemicals from plants as safe and broadly effective alternatives with less

adverse effect. Many beneficial biological activity such as anticancer, antimicrobial, antioxidant, antidiarrheal, analgesic and wound healing activity were reported. In many cases the people claim the good benefit of certain natural or herbal products. However, clinical trials are necessary to demonstrate the effectiveness of a bioactive compound to verify this traditional claim Clinical trials directed towards understanding the pharmacokinetics, bioavailability, efficacy, safety and drug interactions of newly developed bioactive compounds and their formulations (extracts) require a careful evaluation. Hence the major focus of the present investigation is to determine the chemical nature of bioactive principles present and involved in mediating the overall biological activity of ChundaivattralChooranam (CVC) a Siddha Polyherbal formulation. However, it is our contention that searching for biologically active principles of herbal formulation may also afford legitimate new drug leads, and also lead to the isolation of new chemical entities to previously known biologically active substances as well as to compounds with documented non toxicity.

2. Experimental Section

2.1 Plant collection

The botanical species of *Solanumtorvum, Murrayakoenigii, Mangiferaindica, Carunrox burghianum, Phyllanthus embilica, Punica granatum, Trigonella foenumgraecum* were collected from Kolli hills, Salem, India. All of the plant materials are authenticated by botanist and the voucher specimens (SSMC/00/1/1001-7) of the same have been deposited in Department of pharmacognosy for future reference.

2.2 Preparation of the sample

In house formulation of sample was prepared by the method as described in the classical Siddha literature.

In House SOP of CVC.

1. Solanum torvum - 1 part
2. Murraya koenigii - 1 part
3. Mangifera indica - 1 part
4. Carunrox burghianum - 1 part
5. Phyllanthus embilica - 1 part
6. Punica granatum - 1 part
7. Trigonella foenumgraecum -1 part

As mentioned in the text, the above plant parts were collected and dried under shade for 7 days. After drying, each plant material was finely powdered and sieved (40≠). After sieving, equal quantity of each plant powders mixed together. Finally it was stored in airtight container and used for further studies.

2.3 Extraction

The powdered sample of CVC was extracted with 500ml of 90% ethanol by using soxhlet apparatus until the extraction was completed. After the completion of the extraction process, the extract was filtered and the solvent was removed by distillation under reduced pressure. Dark green colour residue was obtained. The percentage yield of the extract was 28 %. It was used for GC-MS analysis.

2.4 Agar-well diffusion assay

The *in vitro* antibacterial activity of Chundaivattral Chooranam (CVC) Siddha Polyherbal formulation was determined by agar- well diffusion method [11]. Log phase bacterial cultures of 10^8 CFU/mL were used. One hundred microlitre of 1 mg/mL concentration were tested against *Pseudomonas aeruginosa* (MTCC No: 4676), *Escherichia coli* (MTCC No: 1588), *Salmonella typhi* (MTCC No: 1167), *Shigella flexneri* (MTCC No: 1457) Diameter of the zone was measured and expressed in mm.

2.5 GC-MS analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the

following conditions: column Elite-1 fused silica capillary column (30 × 0.25 mm ID ×1EM df, composed of 100% Dimethyl poly siloxane, operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 EI was employed (split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C/min, then 5°C/min to 280°C/min, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da. Interpretation on mass spectrum of GC-MS was done using the database NIST08 and WILEY8.The relative percentage amount of each component was calculated by comparing its average peak area to the total areas.

2.6 Fourier Transform – Infra Red Spectroscopy Study (FTIR)

FTIR has proven to be a valuable tool for the characterization and identification of compounds or functional groups (chemical bonds) present in Chundaivattral Chooranam (CVC) herbal extracts [6].The FT-IR spectra of the crude extract samples were recorded on a Thermo Nicolet, Avatar 370 spectrometer equipped with a Deuterated triglycine sulphate detector (DTGS) over the 4000–400 cm−1 range at the resolution of 4 cm−1 and a maximum source aperture. The infrared spectra of the crude extracts were measured (as KBr discs) The important IR bands, such as (C-N), (OH), (C-H), (C=C), (N-H), (C-O) and (C-H) symmetric and asymmetric stretching and stretching frequencies were studied to determine the presence of functional groups in the Chundaivattral Chooranam herbal formulation.

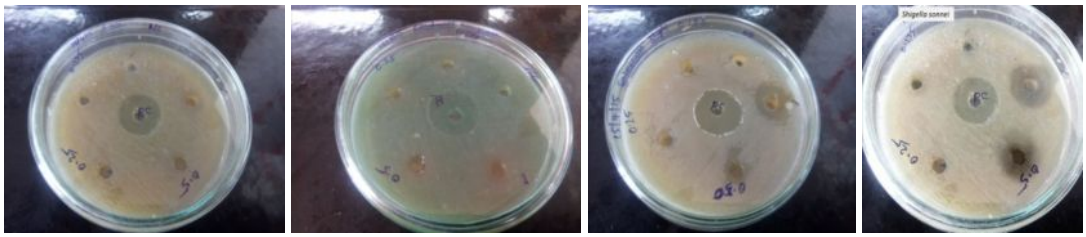
2.7 Statistical analysis

Results were expressed as mean value ± standard error of the mean (SEM) of growth inhibition zones diameters obtained with those CVC which amount was sufficient to perform repetitions.

3. Results and Discussion

The plant contain large amount of primary and secondary metabolites exert a wide range of biological activities on physiological systems [10].In this study three gram -ve organisms were used to determine antibacterial activity. It was observed that Chundaivattral Chooranam herbal formulation (1mg/ml) exerted effective anti-bacterial activity against *Salmonella.typhi*(20± 0.05 mm), *Shigella flexneri* (21± 0.06 mm) when compared to the standard drug Chloramphenicol (1mg/ml) which was evident from the zone of inhibition. However, the inhibition of the test drug was lower than that of standard drug against *Escherichia coli* (8± 0.05 mm), *Pseudomonas aeruginosa* (5± 0.05 mm) (Figure 1). As mentioned with previous studies, 2H-1-benzopyran-6-OL,3,4-dihydro-2,5,7,8-tetramethyl-2-(4-8-12-trimethyl tetradecyl)-2H- -6-yl is a powerful antioxidant and a scavenger of hydroxyl radicals, and it has been shown to have anti-inflammatory activities in tissues [13]. Factors that improve antioxidant status may modulate the immune function; reduce tissue damage and bacterial colonization [5].The investigation was also supported by the previous findings, where 1, 2-Benzenedicarboxylic acid, mono (2-Ethylhexyl) ester are reported with anti-inflammatory and antimicrobial activity [9,12] It also reported that the activities of some plant constituents with compound nature of tocopherols, as antimicrobial, antiinflammatory, antioxidant, hypocholesterolemic, cancer preventive, hepatoprotective, antiarthritic, antihistimic, antieczemic and anticoronary[8].Gas Chromatography-Mass Spectroscopy: GCMS is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, ester, alkaloids, steroids, amino and nitrogen compound. The present research showed the herbal preparation of Chundaivattral Chooranam (CVC) by Gas Chromatography-Mass Spectroscopy. The extracts are a complex mixture of few constituents totally of two major compounds which were identified as 2H-1-benzopyran-6-OL,3,4-dihydro-2,5,7,8-tetramethyl-2-(4-8-12-trimethyl tetradecyl)-2H- -6-yl and 1 2 benzenedicarboxylicacid,Mono(2-Ethyl hexyl)Ester (Figure 2,3). In best of our knowledge and literature survey there is no report of gas chromatography and mass spectrum analysis to identify the chemical compounds from Chundaivattral Chooranam (CVC). The phenomenon of synergistic effects is often crucial to bioactivity in plant extracts and in some cases; the activity is lost in purified fractions. The FTIR spectrum was used to identify the functional groups of the active components present in Chundaivattral Chooranam, based on the peaks values in the region of IR radiation. When the extract was passed into the FTIR, the functional groups of the components were separated based on its peaks ratio. The results of FTIR analysis confirmed the presence of phenol, alkanes, aldehyde, secondary alcohol, amino acid, aromatic amines and halogen compound (Figure4). Development of bacterial resistance to synergistic drug combinations, such as those found in plants, may be

slower than for single drug therapies. It is believed that crude extracts from medicinal plants are more biologically active than isolated compounds due to their synergistic effects 1.The results of this study offer a platform of using ChundaivattralChooranam (CVC) herbal formulation for various diseases including antimicrobial, diabetic, cardiovascular etc.



(a) (b) (c) (d)

Figure 1:Antibacterial activity of ChundaivattralChooranam (CVC) Siddha Polyherbal formulation against (a)*Escherichia coli* (8± 0.05 mm), (b)*Pseudomonas aeruginosa*(6± 0.05 mm),(c) *Salmonella* typhi (20± 0.05 mm), (d) *Shigella flexneri* (21± 0.06 mm)

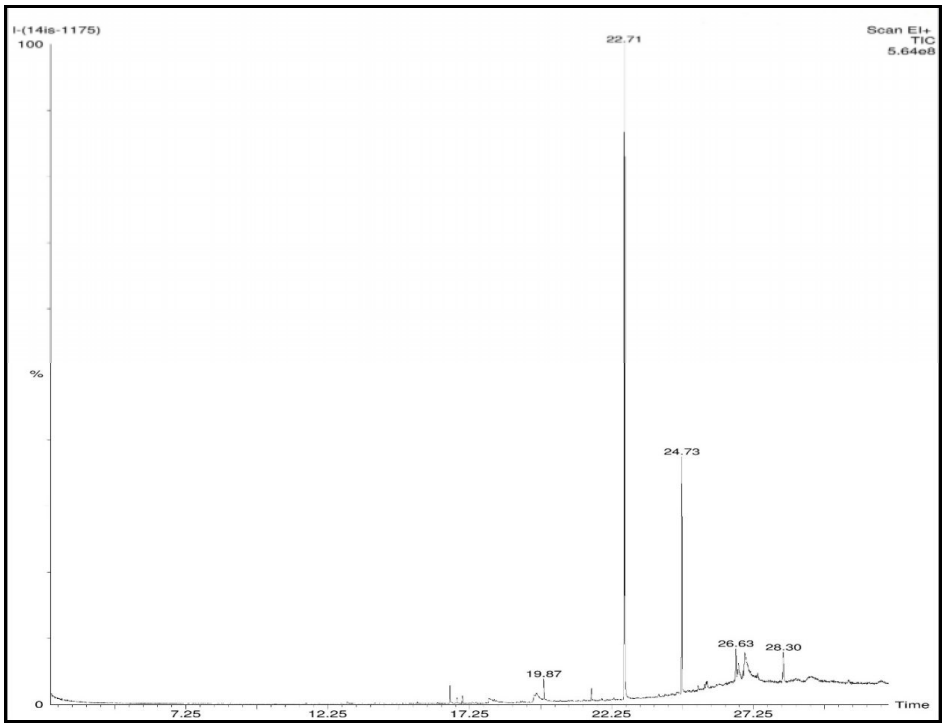
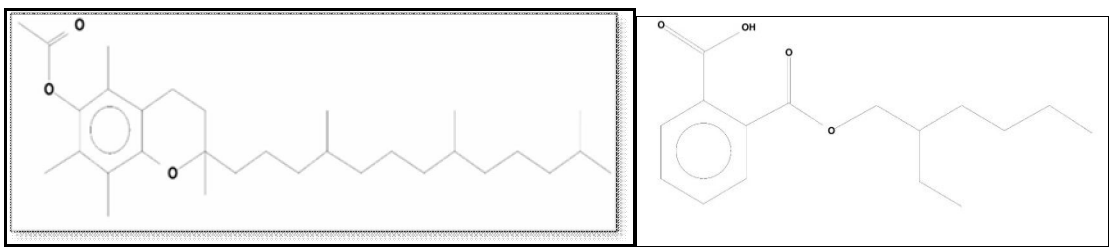


Figure2: The GC-MS chromatogram of the ChundaivattralChooranam (CVC) Siddha Polyherbal formulation.



(a) (b)

Figure 3: Molecular structure and corresponding GC-MS peaks of major bioactive metabolites from Chundaivattral Chooranam (a) 2H-1-benzopyran-6-OL,3,4-dihydro-2,5,7,8-tetramethyl-2-(4-8-12-trimethyl tetradecyl)-2H- -6-yl (MW-410) (b) 1 2 benzenedicarboxylicacid,Mono(2-Ethyl hexyl)Ester (Mw-278)

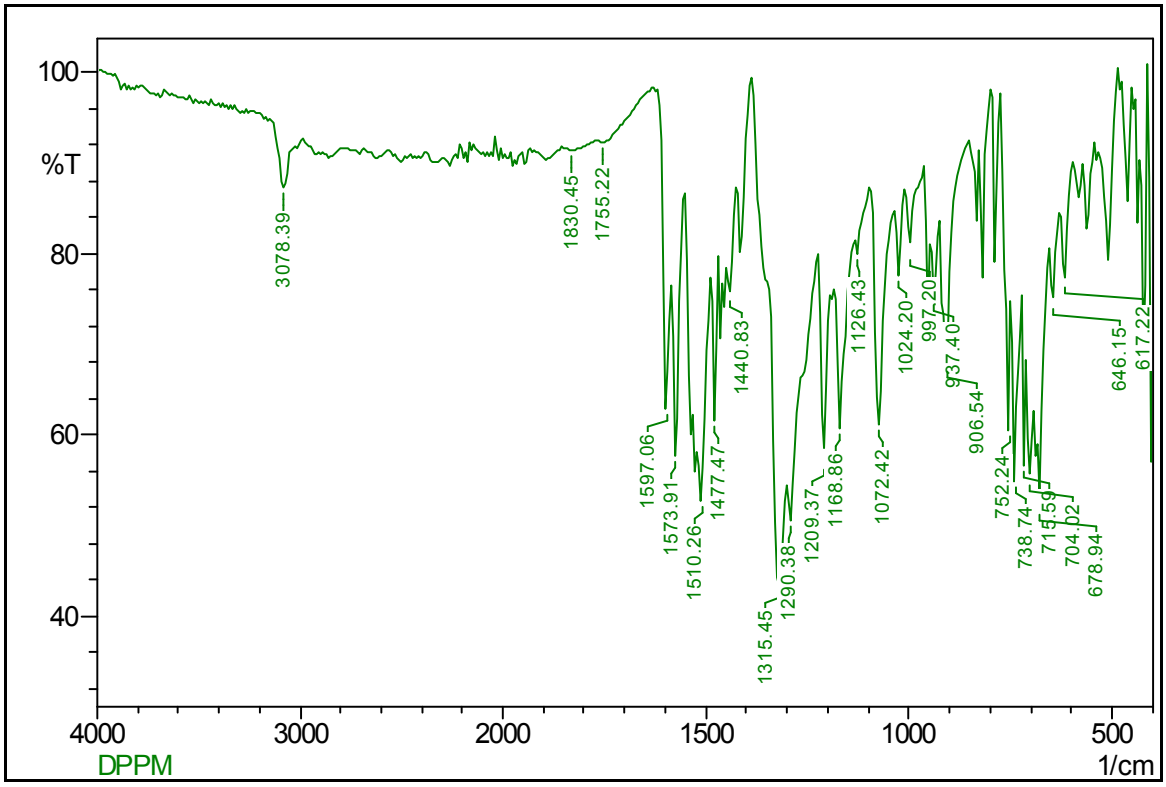


Figure 4: FTIR analysis of the Chundaivattral Chooranam (CVC) Siddha Polyherbal formulation

Conclusion

It was concluded that extract of ChundaivattralChooranam (CVC) is a Siddha Polyherbal formulation possess various potent bioactive compounds and antidiabetic, analgesic, antiseptic, antidysentric, diuretic, antioxidant, antiinflammatory, antiulcer and anticancer properties it is recommended as drug formation to pharmaceutical industries. Further studies are needed to explore the potential bioactive compounds responsible for the biological activities of ChundaivattralChooranam (CVC)

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

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