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Gc-Ms Analysis Of A Poly Herbal Combination From Medicinal Plants Areca Catechu, Acalypha Indica And Piper Betel

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ABSTRACT
InIndian Ayurveda is one of the traditional medicinal systems. The herbal medicine as a form of supporting medicine and also recognize as complete approach in Modern healthcare system. The herbal medicines usages are in trend and it has increasing in a way in the last decade. As a result,World health Organization (WHO) has taken abroader step of including Phytotherapy. Herb-herbcombinations have used in Chinese, Ayurveda andMalaysian traditional medicine practice for manyyears.Many commercial and non-commercialpolyhedral formulations exist in different part ofWorld. Our poly herbal plants ***Areca catechu, Acalyphaindica and Piper betel.***In order to study the chemical characteristic and bioactivity of leaf extracts, a simple and reliable GC-MS method was developed to identify the chemical components from the extract.

Keywords: Ayurveda, Phytotherapy, poly herbal, GC-MS

1. INTRODUCTION

Globally, herbal products have been used as health supplements or therapeutic agents in the treatment of diseases. Herbal medicines remain popular owing to their ease of availability, affordability, and the belief that they are safe for consumption merely because they are plant based or natural.Phytotherapeutic management of pathological conditions encompasses combinatorial intervention of multiple bioactive constituents manifesting multi-target strategy.Polyherbal formulation is also havingmultiple types of molecules against a disease complication.The different molecules cure a disease by different mechanism soprovide a complete therapy against a disease condition.

Areca catechu palm is a stem tall slender, single-trunked palm that has the ability to grow up to 30 meters, the common practice of the plant is by the use of the husk of Areca nut as herbal “chewingsticks” instead of tooth brushes to maintain oral health and hygiene, the seeds are well known for masticatory seed and are used in medicine as well(Jose et al., 2011).The*Areca* nut is the seed of the *Areca* catechu thatgrows mostly in the tropical Pacific, Asia and parts of east

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Africa. The habit of chewing the Areca nutfor its stimulating properties has been used in aboutone tenth of the world's population making it oneof the most consumed psycho active substance. Dried form of the Arecanut was claimed to strengthen gums, sweeten thebreath, eliminate bad taste and act as dentifrices. It has been reported thatthe main components of Areca are polyphenols,fat, polysaccharides and protein. Also the nutscontain alkaloids,arecoline, tannic acid which wassuggested that it can suppress bacteria in the mouth.

An Anting-anting (*Acalyphaindica*) plant is a species of plant having catkin type of inflorescence. This plant traditionally used to treat dysentery, diarrhea, malnutrition, and malarial (Arisandi*et al*, 2008). The activity of anting-anting is related to the chemical constituents such as saponins, tannis, flavonoid, and essential oil (Azmahani*et al*, 2002).

Piper betel belonging to the piperaceaefamily, is one of the precious medicinal herbs found in central and eastern Malaysia, Southeast asia. In India, it is commonly known as paan, which is second to tea and coffee based on daily consumption .Betel leaves are very nutritive and contain substantial amount of vitamins and minerals (Pradhan*et al*.,2013).Widespread use of drugs is leading to the development of resistance against them in the pathogen and also the side effects associated with them is urging people not to use them. Therefore there is a constant and urgent need to develop new antimicrobial drugs for the treatment of infectious disease from medicinal plant.The present investigations, focused on the efficacy of *Areca catechu, Acalyphaindica,* and *Piper betel* and GC-MS method was developed to identify the chemical components from the extract.

2. MATERIALS AND METHODS

Collection of plant material

The *Areca catechu, Acalyphaindica, Piper betel were brought from* Tambarammarket. The plant materials obtained were identified and authenticated by a botanist in the Department of Botany, Pachaiyappa's College, Chennai. The vouchered specimens are deposited at Department of Zoology, Pachaiyappa's College, Chennai – 600 030.

Extraction of various parts of *Areca catechu, Acalyphaindica, piper betel*

The *Areca catechu, Acalyphaindica, piper betel* was cleaned shade dried and coarsely powdered. Successive solvent extraction was done by cold percolation method (Harborne, 1998) by soaking in hexane, chloroform, ethyl acetate, ethanol and methanol successively in an aspirator bottle for 48 h. Aqueous extracts of all the plants were also prepared. After 48 h, the extracts were filtered by Whatman Filter paper No.1. The solvent was removed by distillation using Evator Rotary Evaporator and the extracts were concentrated and dried in Lyodel Freeze Dryer.

Phytochemical studies

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Qualitative analysis of methanol extracts of *Areca catechu, Acalyphaindica, Piper betel* and their poly herbal compound.

Qualitative tests were performed to assess the nature of phytochemicals present in methanol extracts of *Areca catechu, Acalyphaindica, Piper betel* and their poly herbal compounds namely in hexane, chloroform, ethyl acetate, ethanol, methanol and aqueous extracts.

a) Liebermann-Burchard Test: Extract is dissolved in minimum of chloroform. Acetic acid was added and heated. Few drops of acetic anhydride and concentrated H_2SO_4 were added. Green colour shows the presence of Steroid.

b) Noller's Test: Extract is treated with tin and thionyl chloride and was heated in a water bath. Purple colour shows the presence of Triterpenoid.

c) Shinoda Test: Extract is dissolved in alcohol. Magnesium bits and concentrated hydrochloric acid was added. It was heated in a water bath. Majentacolour shows the presence of Flavonoid.

d) Test for Furan: Extract is dissolved in alcohol. p-dimethylaminobenzaldehyde and concentrated hydrochloric acid was added and was heated in a water bath. Pink colour shows the presence of Furanoid compound.

e) Test for Sugar: Extract is treated with anthrone and concentrated H_2SO_4. It was heated in a water bath. Green colour shows the presence of Sugar.

f) Test for Coumarin: Extract is shaken with 10% NaOH. Yellow colour shows the presence of Coumarin. The substance regenerates when concentrated H_2SO_4 is added.

g) Test for Quinone: Extract is treated with concentrated H_2SO_4. Red colour shows the presence of Quinone.

h) Test for Saponin: Extract is shaken with water. Frothing shows the presence of Saponin.

i) Test for Tannin: Extract is shaken with water and lead acetate solution was added. White precipitate shows the presence of Tannin.

j) Test for Acid: Extract is treated with sodium bicarbonate solution. Effervescence shows the presence of Acid.

k) Test for Phenol: Extract is dissolved in alcohol. Ferric chloride is added. Bluish colour shows the presence of Phenol.

l) Test for Alkaloid: Extract is taken in acetic acid and few drops of freshly prepared Dragendorff's reagent are added. A brick red or orange precipitate shows the presence of Alkaloids.

Analysis of the methanol extracts of *Areca catechu, Acalyphaindica, Piper betel* and their poly herbal compound by Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS technique was used in this study to identify the phytocomponents. GC-MS analysis of the fractions was performed using GC-MS-QP 2010 (Shimadzu) and gas chromatograph interfaced to a mass spectrometer (GC-MS) equipped with Elite -1 fused silica capillary column (Length : 30.0 m, Diameter : 0.25 mm, Film thickness : 0.25 μm composed of 100 % Dimethyl poly siloxane). For GC-MS detection, an electron ionization energy system with ionization energy of 70 eV was used. Helium gas (99.999 %) was used as the carrier gas at a constant flow

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rate of 1.51 ml/min and an injection volume of 1 µl was employed (split ratio: 10), Injector temperature 240 °C; Ion-source temperature 200 °C. The oven temperature was programmed from 70 °C (isothermal for 3 min), with an increase of 300 °C for 10 min. Mass spectra were taken at 70 eV; a scan interval of 0.5 sec with scan range of 40 – 1000 m/z. Total GC running time was 35 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a GC-MS solution ver. 2.53.

3. RESULTS AND DISCUSSION

The powdered *Areca catechu, Acalyphaindica*and *Piper betel* were extracted with Hexane, Chloroform, Ethylacetate, Ethanol, Methanol and Aqueous by Soak method. The extractive yields of different solvents of individual plants were presented in the table 1, 2, 3 respectively. And the combined plant extraction of the mentioned plants is presented in the table 4.

The methanolic extracts of the plants *Areca catechu* showed the presence of steroid, triterpenoid, flavonoid, furan, coumarin, sugar, quinine, acid, tannin and phenols , *Acalyphaindica*showed the presence of steroid, triterpenoid, flavonoid, furan, coumarin, saponin, tannin and phenol , *Piper betel* showed the presence of steroids, triterpenoids, flavonoids, phenols, alkaloid, tannin,saponin, quinine, coumarin and furan and PAA showed the presence of steroid, triterpenoid, flavonoid, furan, coumarin, saponin, tannin, and phenols.

Hence the methanol extracts of *Areca catechu, Acalyphaindica, Piper betel* and their ploy herbal compounds were further studied.

GC-MS analysis of methanol extract of *Areca catechu* revealed the presence of five compounds namely methyl salicyclate; o-aminobenzohydroxamic acid, diethyl phthalate; phthalic acid, ethyl isopropyl ester; phthalic acid, ethy pent-2-en-4-yn-1-yl ester (Fig 1). Similarly, the methanol extract of *Acalyphaindica* revealed the presence of six compounds namely methyl salicyclate; phthalic acid, ethyl isopropyl ester; 1,3-dioxolane-2-heptanenitrile, .alpha.-methyl-.delta.-oxo-2-phenylo; .alpha.-d-xylofluranoside, methyl; n-hexadecanoic acid; cyclohexanemethylpropanoate (Fig 2).

Likewise, methanol extract of *Piper betel* revealed the presence of three compounds namely methyl salicyclate; diethyl phthalate; benzoic acid, 2-(1-oxopropyl)- (Fig 3). The methanol extract of PAA ploy herbal compounds revealed the presence of six compounds namely methyl salicyclate; 1,2-benzenedicarboxylic acid, 2-ethyoxy-2-oxoethyl ethyl ester; phthalic acid, ethyl isopropyl ester; benzoic acid, 2-(1-oxopropyl)-; phthalic acid, 4-chloro-3-methylphenyl ethyl ester; phthalic acid, ethy pent-2-en-4-yn-1-yl ester (Fig 4).

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Medicinal plants are a reservoir of biologically active compound with therapeutic properties that overtime have been discovered and used by diverse groups of people for treatment of various aliment of mentioned dental disease. Qualitative analysis and biological activities of *Arecacatechu, Acalyphaindica, Piper betel* and PAA of methanol extracts was studied against the oral pathogens, that causes dental carries. Preliminary screening of the plant such as hexane, chloroform, ethyl acetate, ethanol, methanol and aqueous extract *Arecacatechu, Acalyphaindica, Piper betel* and PAA were found to contain various phyto chemical constituents such as tannins, phenols, alkaloids and flavonoids. Methanolic extract of polyherbal combination of *Arecacatechu* showed a maximum level of phytochemical constituents such as flavonoids, alkaloids,phenols, tannins etc.,.The GCMS analysis revealed the various compounds present in the plant extracts which might exhibit various biological activities.

Phytochemicals present in methanol extract of *Arecacatechu* revealedthe presence of methyl salicylate, o-aminobenzohydroxamic acid, diethyl phthalate, phthalic acid, phthalic acid, ethyl pent-2-en-4-yn-1-yl ester.*Acalyphaindica*were methyl salicylate, phthalic acid, ethyl isoporpyl ester, 1,3-dioxolane-2-heptanenitrile, alpha-methyl-delta-oxo-2-phenylo,alpha-d-xylofuranoside, n-hexadecanoic acid, cyclohexamethylpropanoate.*Piper betel* were methyl salicylate , diethyl phthalate, benzoic acid,2-(1-oxopropyl) and in combination extracts were methyl salicylate,1,2-benzendicarboxylic acid,2-ethoxy-2-oxoethyl ethyl ester,phthalic acid, ethyl isopropyl ester,benzoicacid,phthalic acid,4-chloro-3-methylphenyl ethyl ester,phthalicacid,ethyl pent-2-en-4 yn-1-yl ester.This further showed the efficacy of the extracts .

Table: 1 Qualitative Analysis of extract of *Areca catechu*

S. No	**PHYTOCHEMICAL STUDIES**	**HEXANE**	**CHLOROFORM**	**ETHYL ACETATE**	**ETHANOL**	**METHANOL**	**AQUEOUS**
1	Liebermann-Burchard Test	-	-	-	+	+	-
2	Noller's Test	-	+	-	-	+	-
3	Shinoda Test	-	-	+	-	+	+
4	Test for Furan	-	-	-	+	+	+
5	Test for Coumarin	-	-	-	-	+	+

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6	Test for Sugar	-	-	+	-	+	+
7	Test for Quinone	-	+	-	-	+	-
8	Test for Saponin	+	-	-	+	-	+
9	Test for Acid	-	-	-	-	+	-
10	Test for Tannin	-	-	-	-	+	+
11	Test for Phenol	-	-	+	-	+	+
12	Test for Alkaloid	+	-	-	-	-	-

Table:2Qualitative Analysis of extract of *Acalyphaindica*

S.No	**PHYTOCHEMICAL STUDIES**	**HEXANE**	**CHLOROFORM**	**ETHYLACETATE**	**ETHANOL**	**METHANOL**	**AQUEOUS**
1	Liebermann-Burchard Test	-	-	-	-	+	-
2	Noller's Test	-	+	-	+	+	+
3	Shinoda Test	-	+	-	+	+	-
4	Test for Furan	-	-	-	-	+	-
5	Test for Coumarin	-	-	-	-	+	-
6	Test for Sugar	-	-	+	-	-	+
7	Test for Quinone	-	+	-	-	-	-
8	Test for Saponin	-	-	-	-	+	+
9	Test for Acid	-	-	-	-	-	+
10	Test for Tannin	-	+	-	-	+	+
11	Test for Phenol	-	-	+	+	+	-
12	Test for Alkaloid	-	+	-	+	-	-

Table:3Qualitative Analysis of extract of *Piper betel*

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S. No	PHYTOCHEMICAL STUDIES	HEXANE	CHLOROFORM	ETHYLACETATE	ETHANOL	METHANOL	AQUEOUS
1	Liebermann-Burchard Test	-	-	-	+	-	-
2	Noller's Test	-	-	-	+	+	-
3	Shinoda Test	-	-	+	-	+	+
4	Test for Furan	-	-	-	-	+	+
5	Test for Coumarin	-	-	-	-	+	+
6	Test for Sugar	-	-	-	-	-	+
7	Test for Quinone	-	+	+	-	+	+
8	Test for Saponin	-	-	-	-	+	+
9	Test for Acid	-	-	-	-	-	-
10	Test for Tannin	-	-	-	-	+	+
11	Test for Phenol	+	-	+	+	+	+
12	Test for Alkaloid	-	-	-	-	+	+

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Table: 4 Qualitative Analysis of extract of *Areca catechu,Acalyphaindica, Piper betel* and poly herbal combinations

S.No	PHYTOCHEMICAL STUDIES	HEXANE	CHLOROFORM	ETHYLACETATE	ETHANOL	METHANOL	AQUEOUS
1	Liebermann-Burchard Test	-	-	-	-	+	-
2	Noller's Test	-	+	-	+	+	+
3	Shinoda Test	-	+	-	+	+	+
4	Test for Furan	-	-	-	-	+	-
5	Test for Coumarin	-	-	-	-	+	-
6	Test for Sugar	-	-	-	-	-	+
7	Test for Quinone	-	-	+	-	-	-
8	Test for Saponin	-	-	-	-	+	-
9	Test for Acid	-	-	-	-	-	-
10	Test for Tannin	-	+	-	-	+	-
11	Test for Phenol	-	-	+	+	+	+
12	Test for Alkaloid	-	+	-	+	-	+

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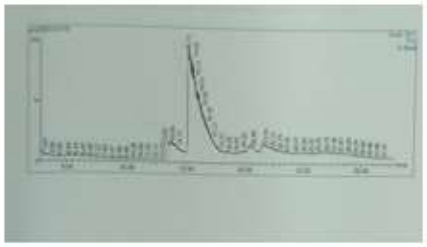
FIGURE 1 GC-MS analysis and phytochemicals present in methanol extract of *Areca catechu*



S. No	Compound Name	Molecular Weight	Molecular Formula
1	METHYL SALICYLATE	152	$C_8H_8O_3$
2	PHTHALIC ACID, ETHYL ISOPROPYL ESTER	236	$C_{13}H_{16}O_4$
3	1,3-DIOXOLANE-2-HEPTANENITRILE, ALPHA-METHYL-, 2(S),5A,-OXO-5-PHENYLO	287	$C_{17}H_{21}O_3N$
4	ALPHA.-D-XYLOFURANOSIDE, METHYL	164	$C_6H_{12}O_5$
5	N-HEXADECANOIC ACID	256	$C_{16}H_{32}O_2$
6	CYCLOHEXANEMETHYL PROPANOATE	170	$C_{10}H_{18}O_2$

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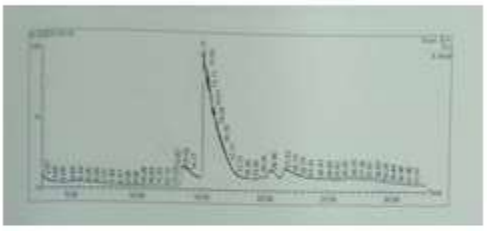
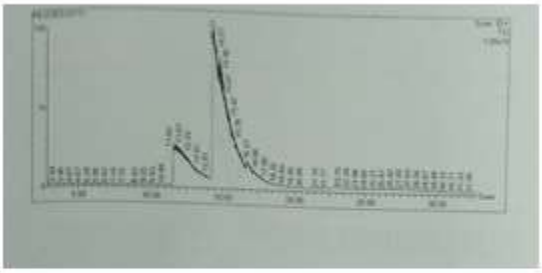
FIGURE 2 GC-MS analysis *and phyotchemicals* present in methanol extract of *Acalypha indica*



S. No	Compound Name	Molecular Weight	Molecular Formula
1	METHYL SALICYLATE	152	$C_8H_8O_3$
2	PHTHALIC ACID, ETHYL ISOPROPYL ESTER	236	$C_{13}H_{16}O_4$
3	1,3-DIOXOLANE-2-HEPTANENITRILE, ALPHA.-METHYL-, DELTA.-OXO-2-PHENYL)-	287	$C_{16}H_{17}NO_3$
4	ALPHA.-D-XYLOFURANOSIDE, METHYL	164	$C_6H_{12}O_5$
5	9-HEXADECANOIC ACID	256	$C_{16}H_{32}O_2$
6	CYCLOHEXANEMETHYL PROPANOATE	170	$C_{10}H_{18}O_2$

FIGURE 3 GC-MS analysis *and phyotchemicals* present in methanol extract of *Piper betel*



S. No	Compound Name	Molecular Weight	Molecular Formula
1	METHYL SALICYLATE	152	$C_8H_8O_3$
2	DIETHYL PHTHALATE	222	$C_{12}H_{14}O_4$
3	BENZOIC ACID, 2-(1-OXOPROPYL)-	178	$C_{10}H_{10}O_3$

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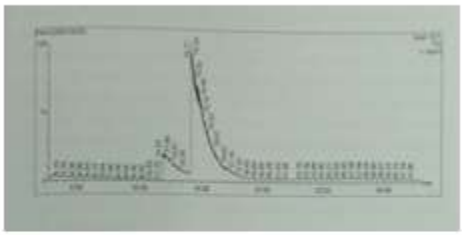
FIGURE 4 GC-MS analysis *and phytochemicals* present in methanol extract of *poly herbal extract*



S. No	Compound Name	Molecular Weight	Molecular Formula
1	METHYL SALICYLATE	152	$C_8H_8O_3$
2	1,2-BENZENEDICARBOXYLIC ACID, 2-ETHOXY-2-OXOETHYL ETHYL ESTER	280	$C_{14}H_{16}O_6$
3	PHTHALIC ACID, ETHYL ISOBORNYL ESTER	234	$C_{18}H_{26}O_4$
4	BENZOIC ACID, 2-(1-OXOPROPYL)-	178	$C_{10}H_{10}O_3$
5	PHTHALIC ACID, 4-CHLORO-3-METHYLPHENYL ETHYL ESTER	318	$C_{18}H_{19}ClO_4$
6	PHTHALIC ACID, ETHYL PENT-2-EN-4-YN-1-YL ESTER	258	$C_{15}H_{14}O_4$

4. CONCLUSION

In the growing countries improved cost of medicine as well astheir side effects has become a great task when the publichealth is concerned. The scientific advancement carries withit the improvement in polyherbal formulations, through thestudy of various phytoconstituents and discovery of usefulherbs combinations which work synergistically to producedesirable effect. Although polyherbal formulation iscommonly used in many parts of the world, but scientificallyit has not been explored.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Indonesia Pharmaceutical Technology Division, Pharmacy Department, Universitas Islam Indonesia

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