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RESEARCH ARTICLE

PHYTOCHEMICAL, ANTIOXIDANT ACTIVITY AND GC-MS STUDY OF POLYHERBAL DRUG FORMULATION

Bharathi J and Hazeena Begum V

Department of Siddha Medicine Tamil University ,
Thanjavur -613 010,Tamilnadu ,India

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Abstract:

The aim of the study was to develop an herbal mixture with medicinal plant varieties such as *Eclipta alba, Spheranthus, Nelumbo nucifera, Occinum sanctum, Aegle marmelous and Centella asiatica*. The phytochemical analysis, DPPH activity and GC-MS was carried out. The study reveals the presence of active ingredients such as Alkaloids tannins, saponins, phenols, flavonoids , glycosides . These bioactive compounds are capable of preventing and fighting oxidative related diseases.

Keywords: **DPPH, GCMS , Vit C**

Introduction

The Indian traditional medicine like Ayurveda, siddha which is largely therapeutic nature has a rich heritage and history. (Patwardhan *et al*., 2009). Even today plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries (Zakaria, 1991). Herbs play a major role in the management of various disorders. Poly-herbal formulations are preferred by the traditional healer than a single herb. The phytotherapists always prefer to prescribe chemically complex remedies and also administer them as complex formulations because the basic fundamental of phytotherapy is that life is chemically complex and so is our food, and therefore the medicines should also be chemically complex (Mills and Bone, 2000). The different plants in the herbal mixture will have different modes of action for curing the disease and in the combined form may sometimes exhibit synergistic activity.

GC-MS is a powerful technique used for many applications which has very high sensitivity and specificity. Generally its application is oriented towards the specific detection and potential identification of compounds based on the molecular mass in a complex mixture. The combination of an ideal separation technique (GC) with the best identification technique (MS) made GC–MS an ideal technique for qualitative and quantitative analysis for volatile and semi-volatile compounds(Cowan 1999). According to World Health Organization about 80% of the world population depends on the natural product for their health due to minimal side effect and cost effective (Jagtap *et al*., 2009).

The preliminary phytochemical studies was carried out using GC-MS analysis in the poly herbal mixture and further supported by in-vitro screening poly phenolic compounds and DPPH scavenging activity.

Materials and methods:

Plant preparation:

The herbal mixture plant such as *Eclipta alba, Spheranthus, Nelumbo nucifera, Occinum sanctum, Aegle marmelous and Centella asiatica* was collected from tamil university. All the plants were shade dried powdered and mixed thoroughly in different proportions. The herbal mixture crude power was added to ethanolic for 18h at room temperature. The extract was filtered and used for phytochemical screening, estimation of total phenols and antioxidant activity.

Phytochemical Screening: Pytochemical tests were studied or analysed on the poly herbal extract using standard qualitative methods as described by Raaman (2006).

Total phenolic content:

The phenolic content was estimated by the method described by Taga *et al* .,1984. Hundred microliter of sample was mixed with 2.0ml of Sodium carbonate (2%) and allowed to stand for 2 minutes at room temperature. After incubation 100 µl of 50% folin-ciocalteau phenol reagent were added then mixed thoroughly and allowed to stand in the dark for 30min at room temperature. Absorbance was measured at 720nm using UV-visible spectrophotometer (Shimadzu, UV-1800). The phenolic contents were expressed as caffeic acid equivalent per gram (CE/g)

DPPH Radical Scavenging assay:

It is one of the most extensively used antioxidant assay for plant samples. This assay is based on the measurement of the scavenging ability of antioxidant test substances towards the stable radical. The free radical scavenging activity of aqueous ethanolic extracts was examined *in vitro* using DPPH radical. (Tepe, & Sokmen, 2007). The test extracts were treated with different concentrations from a maximum of 250 µg/ml to minimum of 4 µg/ml. The reaction mixture consisted of 1 ml of 0.1mM DPPH in ethanol, 0.95 ml of 0.05 M Tris-HCl buffer (pH 7.4), 1 ml of ethanol and 0.05 ml of the herbal extract. The absorbance of the mixture was measured at 517 nm exactly 30 sec after adding extract.

DPPH radical scavenging that is calculated by the Formula:

$$\% \text{ DPPH radical scavenging} = \frac{\text{O.D of control} - \text{O.D of test}}{\text{O.D of control}} \times 100$$

Ascorbic acid is used as a positive control.

GC-MS analysis:

Preparation of extract:

20 g of the herbal mixture were soaked in 95% ethanol for 12 h. The extracts were then filtered through Whatman filter paper No.1 125mm along with 2 gm sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with 95% ethanol. The filtrate was then concentrated by bubbling nitrogen gas into the solution. The extract contained both polar and non-polar phytocomponents of the plant material used. 2 µl of these solutions was employed for GC/ MS analysis .

GC analysis:

GC-MS analysis was carried out on a GC CLARUS 500 PerkinElmer system comprising a 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, then 5°C/min to 280°C, 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 .

RESULT AND DISCUSSION:

SCREENING OF PHYTOCHEMICAL ANALYSIS

The Poly Herbal analysis subjected to preliminary qualitative chemical analysis (Raman, 2006).The preliminary Phytochemical analysis was conducted for the presence of bioactive compounds such as alkaloids, glycosides, tannins, saponins, flavonoids , phenolic compounds and vitamin C (Table 1).

TOTAL PHENOL AND DPPH RADICAL SCAVENGING ACTIVITY:

Poly phenolics compounds are well known as antioxidant and scavenging agents against free radicals associated with oxidative damage (Fergusion *et al* ., 2006) .The presence of these compounds in may give credence to usage for the management of oxidative stress induced ailments. Antioxidative properties of poly phenols arise from their high reactivity as hydrogen or electron donors from the ability of the poly phenol derived radical to stabilize and delocalize the unpaired electron (Chain- breaking function) and from their potential to chelate metal ions (termination of the fenton reaction) (Rice –Evans *et al* .,1996). In the present study, the analysis of herbal mixture was found to be contain rich source of Poly phenols (86.2±8.3 mg/100ml) The highest value of phenolic content indicates that the plant has high antioxidant activity (Table 2).

In the present study several free radical scavenging activities of Poly herbal mixture extract *were* evaluated by DPPH scavenging assay. Poly herbal mixture extracts have got profound antioxidant activity. DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, which gets decolorized in the presence of antioxidants (Burits, and Bucar ,2000) The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, it gets decolorized which can be quantitatively measured from the changes in absorbance at 517nm.The Poly herbal mixture exhibited a significant dose dependent inhibition of DPPH activity. In this study, at 125 µg /ml, the extract showed highest inhibition of DPPH activity shown in (Table 3).The results of DPPH-free radical scavenging assay suggest that the Poly herbal mixture extract extract is more capable of scavenging free radicals.

GC-MS STUDY

The presence of phytochemicals in the ethanolic extract of Poly herbal Formulation is tabulated .Seventeen compounds were identified in the extract by GC-MS analysis(Table 4).The active principles with their Retention Time (RT),Molecular Formula ,Molecular Weight and Concentration (%) are found . The major components present in the poly herbal mixture such as 1,3-Benzenediol , Conessine , Ergost-7-en-6-one, Estran-17-one, 4H-1-Benzopyran-4-one, 5-Hydroxy-3',4',6,7,- Tetramethoxy flavone and 2H- Benzimidazol-2-one. The result indicate the plant possess potential antioxidant properties.

Conclusion:

Different phytochemicals have been found to have a broad range of activities, which may help in protection against chronic diseases. Antioxidant properties, particularly radical scavenging activities, are very important due to the harmful role of free radicals in foods and biological systems. The reduction of DPPH absorption is indicative of the capacity of the extracts to scavenge free radicals, independently of any enzymatic activity .Alkaloids and flavonoids are the major phytochemicals found in herbal mixture . Flavonoids ,tannins plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. The compounds identified by GC-MS in ethanolic extract are medicinally valuable and which exhibits antioxidant behavior. So our results indicate the poly herbal formulation has potential antioxidant properties of therapeutic for the treatment of diseases. The confirming our study on relevant animal models followed by the clinical support in future for development of poly herbal formulation.

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Table 1:The analysis of phytochemicals in the Herbal Mixture Plant Extract

Alkaloids	Glycosides	Tannins	Saponins	Phenolic compounds	Flavonoids	Vitamin
+	+	+	+	+	+	+

Table 2:Total Phenol content of Poly Herbal Mixture

Phytochemical Constituents	Concentration (mg/100g)
Total Phenol	86.2±8.3

Table 3:DPPH Radical Scavenging Activity of Poly Herbal Mixture

Drug	4 µl/ml	8 µl/ml	15 µl/ml	30 µl/ml	60 µl/ml	125 µl/ml
Poly Herbal Formulation	27.02 ± 0.002	29.31± 0.002	31.85 ± 0.001	33.31 ± 0.001	45.44 ± 0.002	47.03 ± 0.002
Vit C	**0.1 µl/ml**	**0.2 µl/ml**	**0.4 µl/ml**	**0.6µl/ml**	**0.8 µl/ml**	**1 µl/ml**
	5.82 ± 0.002	14.12 ± 0.001	28.21 ± 0.001	45.18 ± 0.003	61.25 ± 0.001	77.12 ± 0.001

Table.4. GC-MS: Phytocomponents in methanolic extract of Caralluma fimbriata by GC-MS Report

No.	RT	Name of the compound	Molecular formula	MW	Peak Area %
1.	3.56	1,3-Benzenediol, 5-iodo-	$C_6H_5IO_2$	236	6.16
2.	5.65	10H-1,3-Dioxolo[4,5-b]xanthen-10-one, 11-methoxy-	$C_{15}H_{10}O_5$	270	6.85
3.	7.02	3-oxo-16-demethoxycarbonyl-16-(2-methyl-sulphinylacetyl)-20-desethyl-20-allylvincadifformine	$C_{23}H_{26}N2O_3S$	410	6.85
4.	8.28	2-(2-Bromobenzoylamino)benzothiazole	$C_{14}H_9BrN_2OS$	332	6.85
5.	10.04	Conessine	$C_{24}H_{40}N_2$	356	4.79
6.	12.72	Ergost-7-en-6-one, 2,3,14-trihydroxy-, (2á,3á,5á,24R)-	$C_{28}H_{46}O_4$	446	4.11
7.	14.66	Estran-17-one, 3-hydroxy-, (3à,5à)-	$C_{18}H_{28}O_2$	276	6.85
8.	15.87	4H-1-Benzopyran-4-one, 3-(2,4-dimethoxyphenyl)-5-hydroxy-7-methoxy-	$C_{18}H_{16}O_6$	328	6.16
9.	17.87	5-Hydroxy-3',4',6,7-tetramethoxyflavone	$C_{19}H_{18}O_7$	358	5.48
10.	21.55	Cirsilineol (5,4'-dihydroxy-6,7,3'-trimethoxyflavone)	$C_{18}H_{16}O_7$	344	6.85
11.	22.46	4-Methyl-6-phenyl-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2H)-one #	$C_{10}H_9N_3OS$	219	6.85
12.	25.82	2H-Benzimidazol-2-one, 1,3-dihydro-5-methyl-	$C_8H_8N_2O$	148	6.85
13.	28.28	5-Cyano-2-methyl-4-methylthio-6-phenylpyrimidine	$C_{13}H_{11}N_3S$	241	6.85
14.	29.82	2,5-Bis(4-biphenylyl)-1,3,4-oxadiazole	$C_{26}H_{18}N_2O$	374	3.42
15.	32.56	2-p-Chlorobenzoyl-2-anilthioaceticacid anilide	$C_{21}H_{15}ClN_2OS$	378	4.79
16.	33.99	Benzamide, N,N-dimethyl-4-(4-methyl-1-phthalazinylamino)-	$C_{18}H_{18}N_4O$	306	6.16
17.	35.73	1-Propene, 1,3,3-trifluoro-1,3-bis(methylthio)-2-(trifluoromethyl)-	$C_6H_6F_6S_2$	256	4.11