Research Article

The GC-MS and Antioxidant Study of an Ayurvedic Medicine Ayaskriti

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ABSTRACT

Ayskriti is one Ayurvedic medicine used for the treatment of anemia, skin diseases and weight loss. The present study deals with the GC MS analysis and antioxidant studies namely, reducing power, POD and Catalase assays of Ayaskriti. The GC MS analysis indicated the presence of some important medicinally important bio molecules such as eucalyptol, eugenol, phenylethyl alcohol, ethyl hydrogen succinate etc. among other compounds. Antioxidant activity was evident in all the three assays. Further studies are required to prove the medicinal efficacy of this medicine.

Keywords: Ayaskriti, Ayurvedic, GC MS, POD, Catalase, Eugenol, Antioxidant.

INTRODUCTION

Ayurveda is one of the oldest forms of medicinal practice which dates back to the Vedas. It has come along and stood the test of time. In the present scenario of medicinal practice the role of Ayurveda is all the more crucial since it is more natural, easily accessible and affordable. But due to the lack of information on the scientific efficacy by standard procedures its acceptability always creates lots of controversies and debate. It is high time to change this trend to establish Ayurveda, Sidha and other forms of traditional medicines as scientifically validated and proven forms of treatment. Some reports in direction are already there and the list is increasing as time passes and this should continue.[1-19]

Ayaskriti is an Ayurvedic medicine which is used to cure anaemia, weight loss, skin diseases etc. It contains iron as one of the ingredient. The word Ayas in Sanskrit means Iron. This medicine is made of a number of medicinal plants and plant parts along with metallic iron. Ayskriti is made of the following ingredients which are divided into three sections: One part is known as Kwatha Dravyas, the second is Sandana Dravyas and the third is Prakshepaka dravyas. All the ingredients of Kwatha dravyas are taken at 960 grams each and mixed with 98 litres of water. The ingredients of Sandana dravyas are 9.5 kg of Jaggery and 1.6 litres of Honey. The Prkshepaka dravyas are taken at 48 grams each including pure iron foil.

The ingredients areAsana (*Pterocarpus marsupium*), Tinisha (*Anogeissus lactifolia*), Bhurja (*Betulautilis*), Swethavaha (*Calotropis procera*), Prakeerya (*Sapindus trifoliatus*), Khadira (*Acacia catech*), Kadara (*Acacia polyentha*), Bhandi (*Rubia cordifolia*), Shimshapa (*Dalbergia sisoo*), Meshashrungi (*Prosopis spicegera*), Chandana (*Santalum album*), Raktachandana (*Pterocarpus santalinus*), Daruharidra (*Berberis aristata*), Tala (*Borassus flabellifer*), Palasha (*Butea monosperma*), Jonkaka (*Aquilarisa gallocha*), Shaka (*Grewia populifolia*), Shala (*Shorea robusta*), Kramuka (*Phyllanthus reticulates*), Kalinga (*Holarrhena antidysenterica*), Ajakarna(*Acacia leucophloea*), Aswakarna (*Cassia fistula*) and water 98 litres.Kalinga (*Holarrhena antidysenterica*), Murva (*Marsdenia tenacissima*), Bharangi (*Clerodendron serratum*), Katuki (*Picrorrhiza kurroa*), Maricha (*Piper nigrum*), Ativisha (*Aconitum heterophylum*), Gandira (*Coleus forskohlii*), Ela (*Elettaria cardamomum*), Pata (*Cycleapelteta*), Ajaji (*Carumcarvi*), Kadvangaphalam (*Oroxylum indicum*), Ajamoda (*Trchyspermum roxburghianum*), Sarshapa (*Brassica alba*), Vacha (*Acorus calamus*), Jeeraka (*Cuminum cyminum*), Hingu (*Ferula foetida*), Vidanga (*Embliaribes*), Pasugandha (*Cleome gynandra*), Pippali (*Piper longa*), Pippalimoola (Root of *Piper longa*), Chavya (*Piper retrofractum*), Chitraka (*Plumbago zeylanica*), Shunti (*Zingiber officinale*), Shudha lohapatra (Pure iron foil).

Not much is done so far on the various pharmacological parameters of Ayskriti to prove its efficacy. We have reported the antioxidant properties of Ayaskriti at a preliminary level (Lenin *et al*, 2016) and this is the second report to understand the medicinal efficacy by standard methods.[20]

The GC MS analysis of Ayaskriti by standard procedure indicated the presence of some very important bio molecules which have medicinal activities similar to that of Ayaskrti. The antioxidant study of Ayaskriti also revealed its medicinal role.

MATERIALS AND METHODS

The medicine Ayaskriti was procured from a standard Ayurvedic vendor at Chennai, India. This was subjected to GC MS analysis following necessary protocols. The antioxidant assays, namely, reducing power formed by assay, POD assay and Catalase assay was per standard procedures.

GC-MS Analysis

The medicine was subjected to GC-MS analysis by standard method after processing it suitably. The metabolites in the samples were identified using a P2010 gas chromatography with thermal desorption system TD20 coupled with mass spectroscopy (Shimadzu). The ionization voltage 70ev and GC was conducted in the temperature programming mode with a Retek column (0.25mm, 60m, XTI-5). The temperature in the initial column was 80^0c for 1 min, and then increased linearly to 70^0c to 220^0c held for 3 min followed by linear increased temperature 100^0 c up to 290^0c and held for 10min. The injection port temperature was 290^0 c and the GC-MS interface was maintained at 29^0c, the samples were introduced via an all glass injector working in the split mode with helium carrier gas low rate with 1.2 ml per minute. The identification of metabolites was accomplished by comparison of retention time and fragmentation pattern with mass spectra in the NIST spectral library stored in the computer software (version 1.10 beta, Shimadzu) of the GC-MS. The relative percentage of each extract constituent was expressed with peak area normalization.

Antioxidant Test

Preparation of sample

1gm of sample were weighed and dissolved in 10ml of distilled water and filtered. The concentration of drug was 0.1, 0.5, and 1ml respectively.

Reducing power assay

The reducing power of the herbal medicine extract was determined by a slightly modified method of (Oyaizu 1986).[21]The reducing ability of the drug extract was measured by the transformation of Fe^{3+} toFe^{2+} in the presence of the extract at 700nm. Increased absorbance of the reaction mixture indicates increased reducing power. 1 ml of medicine sample concentration (0.1, 0.5 and 1 mg/ml) was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and Potassium ferricyanide [K_3Fe (CN) $_6$] (2.5 ml, 1 %). The mixtures were then incubated at 50°C for 20 min. Aliquots (2.5 ml) of Trichloroacetic acid (10 %) were added to each mixture, which were then centrifuged for 10 min at 1000 rpm. The upper layer of the solutions (2.5 ml) were mixed separately with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1 %), and the absorbance levels were measured at 700 nm using a spectrophotometer.

Guaiacol Peroxidase (POD) assay

Peroxidase activity was determined according to Panda *et al,* (2003)with slight modification.[22] Each solution were treated with 2ml of a solution containing Guaiacol, H_2O_2 and phosphate buffer (pH 7) in the concentrations of 1%, 40mM and 100mM, respectively. The enzyme produced a colourful product by using H_2O_2 and Guaiacol as substrates. The absorbance of the product was monitored at 470 nm and peroxidise activity was indicated in the form of graph.

Catalase (CAT) assay

Catalase activity was determined according to (Aebi and Lester (1984).[23] The decomposition of H_2O_2 was followed as a decrease in absorbance at 240 nm in a UV/Vis spectrophotometer. 50 mm potassium phosphate buffer, pH 7.0 and 10mM H_2O_2 mixture was used. The extinction coefficient of H_2O_2 (40 mM−1 cm−1 at 240 nm) was used to calculate the enzyme activity that was expressed in terms of milli moles of H_2O_2 per minute per gram fresh weight.

RESULTS AND DISCUSSION

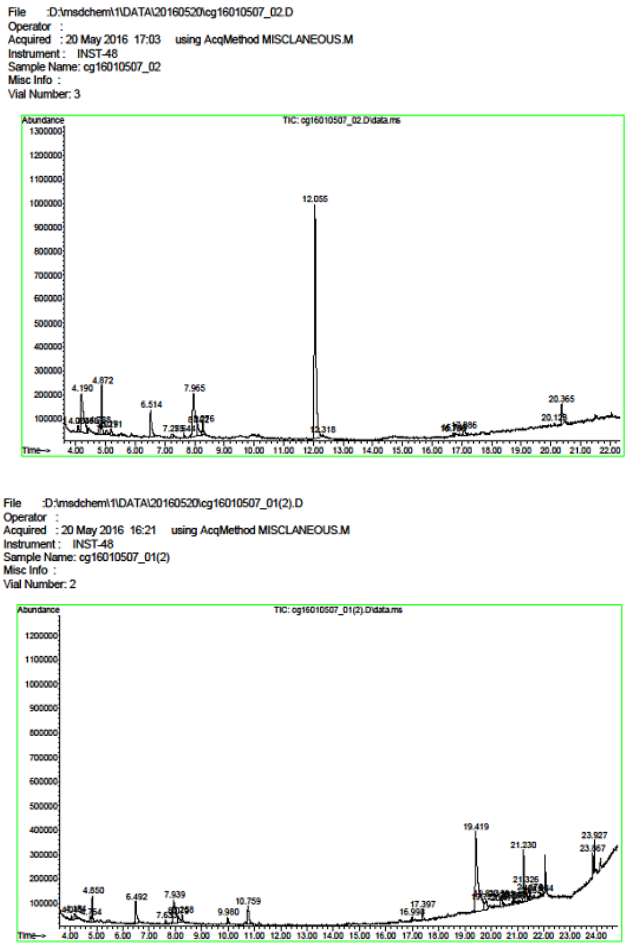

Figure 1 and Figure 2: Show the GC Ms graphs of Ayaskriti

Table 1: Indicates the GC MS Analysis Study of Ayaskriti. The important Retention times, Peaks % values and

probable compounds as compared with D: \Database\NIST05a.L.

S. No.	Peak Area %	RT	Probable Names of compounds
1	1.18	4.0621	Butane, 1-(1-methylethoxy)- 1-Propanol, 2-ethoxy- 12-[Trifluoromethyl]-3,6,9-trioxatridecan-1-ol
2	4.191	12.95	Glycerin
3	4.790	1.79	Propane, 2-cyclopropyl- 4-Methyloxazolidine Butanoic acid, 2-oxo-
4	4.873	5.51	Eucalyptol
5	5.190	1.14	Butanoic acid, 3-hydroxy-, methyl ester Propanol, methoxy-, acetate
6	6.517	5.24	Phenylethyl Alcohol
7	7.967	13.97	Ethyl hydrogen succinate 4-Ketopimelic
8	8.102	2.63	2-Oxazolidinone, 3-methyl-
9	8.278	1.72	Oxirane, [(1-methylethoxy)methyl]- p-menth-1-en-8-ol 3-Cyclohexene-1-methanol, .alpha., .alpha.4-trimethyl-
10	12.053	44.79	Phenol, 2-methoxy-3-(2-propenyl)- 3-Allyl-6-methoxyphenol Eugenol
11	17.085	1.08	N-Acetyl-.alpha.-methyl-4-ethynyl- 2-thiazolemethanamine 2,4'-Dihydroxy-3'-methoxyacetophen one Imidazo(1,2-a)pyrimidine, 6-methyl -5-oxo-1,2,3,5-tetrahydro-
12	20.367	4.09	Phenazine, 1-methoxy- Dodecanoic acid, 1,1'-biphenyl-4-y lcarbonylmethyl ester 2-Fluorenamine

The GC MS analysis has indicated the presence of some important bio molecules which have high medicinal values such as Glycerin, Eucalyptol, Phenylethyl alcohol, Eugenol, Butanoic acid, 2-oxo-, Ethyl hydrogen succinate etc. Glycerin is a common chemical used for cosmetic application for smoothness of skin. Eucalyptol is used in mouth washes and cough suppressants. Phenylethyl alcohol has antibacterial properties. Eugenol has been reported to have many important medicinal properties as is described by many reporters. It is an antifungal agent particularly against *Candida albicans*.[24,25] Eugenol is a powerful fat soluble antioxidant and maintains the activities of the body antioxidant enzymes.[26] Pharmacologically eugenol has been reported as anticonvulsant and local anaesthetic, antistress and bacteriostatic and bactericidal.[27,28,29] Rompelberg*et al*, 1996, have demonstrated the effect of eugenol on the genotoxicity of established mutagens in liver.[30] Anticarcinogenic potential of Eugenol was reported by Zheng*et al*, 1992.[31] It also depresses the activity of central nervous and neuro muscular function.[32] It also prevents radiation induced chemical oxidative damage in cell membranes and modify the membrane associated signaling process after radiation exposure.[33] Ethyl hydrogen succinate is used to prepare antibiotics.

The results of antioxidant studies are shown below.

Table 2: Indicates the reducing power activity reading of Ayaskirti.

Conc.	0.1(sample)	0.5(sample)	1(sample)
200	4	4	3.961
300	4	4	4
400	4	4	2.36
500	0.575	0.252	0.655
600	1.458	0.285	1.604
700	1.965	0.368	2.222

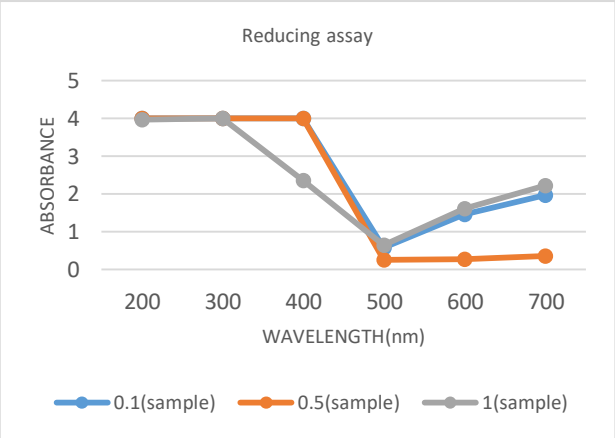

Figure 3: Indicates the graph for reducing power assay at different Concentration of sample.

Table 3: Indicates the peroxidase activity reading of Ayaskirti.

Wavelength	Concentration		
	0.1 mg/ml	0.5 mg/ml	1.0 mg/ml
200	4	3.28	3.941
300	2.514	0.718	4
400	0.8	0.184	1.985
500	0.318	0.072	0.83
600	0.187	0.032	0.472
700	0.097	0	0.281

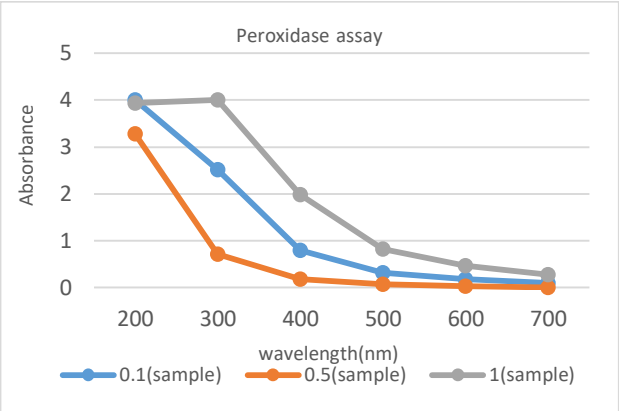

Figure 4: Indicates the POD assay activity of Ayaskirti

Table 4: Indicates the catalase activity reading of Ayaskirti.

Conc.	0.1(sample)	0.5(sample)	1(sample)
200	3.948	3.999	3.975
300	1.223	1.196	3.99
400	0.054	0.023	1.095
500	0.003	0.001	0.516
600	-0.003	-0.001	0.345
700	-0.004	-0.003	0.262

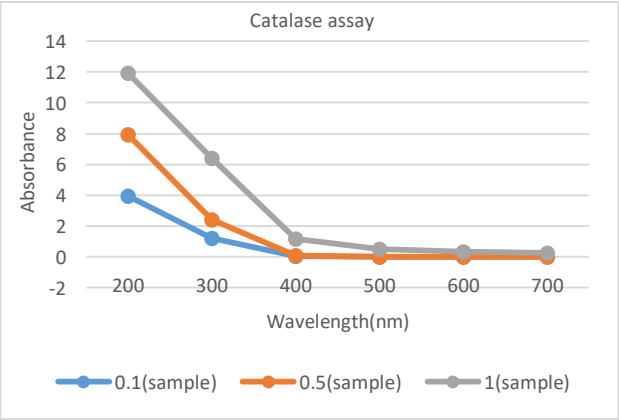

Figure 5: Indicates the Catalase activity of Ayaskirti.

The reducing power assay (Table 2 & Figure 3), peroxidase assay (Table 3 & Figure 4) and catalase assay (Table 4 & Figure 5) of Ayaskirti indicated maximum activity at 200 and 300 unit concentration of 1 ml, 0.1 ml and 0.5 ml water solution whereas the concentrations increased the activity reduced. These results could be help full in fixing the dose of medicine along with concentration.

It is interesting that the Ayurvedic proponents have used so many plants, plant parts and Iron for the preparation of Ayaskriti. It is quite intriguing as to the choice of the ingredients, their proportion and the methodology for preparation. This indicates their deep knowledge and understanding of the medicinal values of each ingredients separately and also that of the final product after due processing. It is high time to probe into these intricacies of Ayurveda to bring this great science of medicine to the fore. The present work is a humble step in this direction. Further work is in progress to establish the pharmacological, toxicological and other similar important parameters to prove the efficacy of Ayaskriti.

CONCLUSION

The above findings indicate that Ayaskriti contains some important bio molecules of medicinal importance through GC MS analysis. The antioxidant potentials of Ayaskritiis also of great medicinal importance in the mechanism of action of this medicine. To prove the efficacy of this medicine these are the initial steps and further work is warranted and it is the process.

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