Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 2, 2012

Full Proceeding Paper

CHEMICAL STANDARDIZATION STUDIES ON RASAGANTHI MEZHUGU

***SUDHA V, LAVANYA R, NIRAIMATHI K L, SAVARIRAJ SAHAYAM C, BRINDHA P ***

Centre for Advanced Research in Indian System of Medicine, SASTRA University, Thanjavur, TamilNadu, India. Email: brindhajana@yahoo.co.in

Received: 16 March 2012, Revised and Accepted: 20 April 2012

ABSTRACT

Ayurvedha and siddha are two complementary indigenous systems of medicine contributing significantly towards the health care of human society. Lack of standardization and validation is a lacuna in these herbals products causing apprehension on their safety and efficacy. In the recent years there has been considerable research on traditional medicines focusing towards this validation and standardization aspects. In the present work, Rasaghanthi mezhugu used to treat many oxidative stress related disease is chemically evaluated for its chemical potentials. This formulation has been subjected to physicochemical standardization and identification of active constituents employing modern instrumentation techniques like HPTLC finger printing and GC-MS analysis. The data of the results obtained are presented and discussed. Such studies will contribute not only towards production of standard traditional medicines leading to international recognition and acceptance, but also in increasing their market potentials.

INTRODUCTION

Recently there is an increasing trend observed for traditional medicine based research towards providing modern scientific proof and also to obtain leads for the development of certain plant related medicines.

The Rasaganthi mezhugu, a unique siddha preparation composed of 38 herbal plants and 8 metals including Elemental Mercury, Elemental Sulphur, Mercurous chloride, Arsenic trisulphide, iron, Zinc, Copper Sulphate and Lead monoxide. Rasagandhi mezhugu in the form of capsule is used (1).This formulation is also used in the treatment of all Skin diseases, piles, various kinds of neuralgic pains, glandular enlargements, and various kinds of cancerous affections.

Realizing the significant importance of this herbomineral preparation, an attempt is made in the present work to determine the chemical standards for this preparation.

MATERIALS & METHODS

Rasagandhi mezhugu is purchased from SKM Siddha, Erode. The formulation appeared as a semisolid mass.

Ingredients of the Rasagandhi mezhugu:

Table A: Botanical origin

S. No	Siddha Name	Scientific Name
1	Chukku	Zingiber officinale Roscoe.
2	Omam	Trachyspermum ammi L.
3	Manjal	Curcuma longa L.
4	Vaividangam	Embelia ribes Burm.f.
5	Vasambu	Acorus calamus L.
6	Lavangam	Cinnamomum zeylanicum(Bl.)
7	Parangi pattai	Cucurbita pepo L.
8	Seran kottai	Semecarpus anarcadium L.
9	Kadukkai thole	Terminalia chebula Retz.
10	Karum seeragam	Nigella sativa L.
11	Kaattu seeragam	Centratherum anthelminticum L.
12	Siruthekku	Premna herbacea Roxb.
13	Thaaleesapathiri	Taxus baccata L.
14	Thiraatchai	Vitis vinifera L.
15	Thippili	Piper longum L.
16	Sitraraththai	Alpinia speciosa L.
17	Kottam	Saussurea lappa Clarke
18	Valuluvai arisi	Celastrus paniculatus Willd.
19	Perum seeragam	Foeniculum vulgare Miller.
20	Ela arisi	Elataria cardamomum L.
21	Jadhikkai	Myristica fragrans Houtt.
22	Milagu	Piper nigrum L.
23	Seeragam	Cuminum cyminum L.
24	Karboga arisi	Psoralea corylifolia L.
25	Maasikkai	Quercus infectoria oliver
26	Thippili moolam	Piper longum L.
27	Pirappan kizhangu ver	Calamus rotang L.
28	Ettikottai	Strychnos nux –vomica L.
29	Thetraankottai	Strychnos potatorum L.
30	Neermulli vidhai	Asteracantha longifolia L.Nees
31	Yellu	Sesamum indicum L.
32	Kopparai thenkai	Cocos nucifera L.
33	Kollu	Dolichos biflorus L.
34	Siru chinni ver	Acalypha fruticosa Forsk.
35	Mutchangan ver	Azima tetracantha Lam
36	Amukkara kizhangu	Withania somnifera L.
37	Aaakaasa garudan kizhangu	Corallocarpus epigaeus Rotrl.
38	Kodiveli ver pattai	Plumbago rosea L.
39	Kozhimuttai	Gallus domesticus L.
40	Panai vellam	Borassus flabellifer L.

Table B: Metal and Mineral origin

1	Rasam	Elemental Mercury
2	Gandhakam	Elementary Sulphur
3	Pooram/Rasa karpooram	Mercurous chloride
4	Paththra thalagam	Arsenic trisulphide
5	Kaantham	Magnetite ore of Iron
6	Thurusu	Copper sulphate
7	Paal thutham	Zinc carbonate with traces of Zinc sulphate
8	Mirudarsingi	Lead monoxide

Methodology

Physicochemical standardization:

The Physico-chemical characterization studies including pH, Loss on drying at 105° C, Total ash, Acid Insoluble ash and determination of extractive values were carried out according WHO Guidelines. Quantitative estimation of phytoconstituents such as Alkaloids, flavanoids, terpenoids, saponins and total fatty matter were also carried out.

Determination of Microbial Load

The microbial loads were determined as per Ayurvedic Pharmacopoeia.

HPTLC analysis

HPTLC finger printing profile was determined for alkaloids and flavanoids (3, 4).

Sample preparation for alkaloids and flavanoids

2.0094g of the sample was weighed in a 250ml beaker. Extracted with 75ml of methanol by boiling on water bath for about 20min and the extract were transferred to 250ml beaker.

The process was repeated for 4 to 5 times till the raw material is completely extracted or till the extract becomes colorless. Collected the extracts and concentrated to below 10ml. The concentrated extract is filtered off and is taken for TLC analysis.

Instrumentation and Chromatographic conditions

Methanol extracts of Rasagandhi mezhugu were spotted on a 10x10cm Silica gel 60 F_{254} precoated plate (E.merck) of thickness 0.2mm, 7mm wide band using automatic TLC applicator Linomat V and 8mm from the bottom. The mobile phase used for alkaloids was Toluene: Ethyl acetate: Diethyl ether (60:30:10 v/v/v) and for flavanoids was Toluene: Ethyl acetate: Acetic acid: Methanol (2.5:7.0:0.25:0.25 v/v/v/v). The plates were eluted by ascending mode to 8cm in a CAMAG TLC chamber which was saturated with the mobile phase vapour for 20min before elution. The plates were dried after development and then scanned at 345nm for alkaloids and 280nm for flavanoids (Quercetin as a standard) by using CAMAG Scanner3 with WINCAT software version 1.3.4. The plates were photographed at 254nm and 366nm using CAMAG Reprostar3 (Fig 1&2).

GC-MS analysis

GC-MS analysis of acetone extract was done on a GC clarus 500 Perkin Elmer system interfaced to mass spectrometer (GC-MS) instrument employing standard protocols (5).

RESULTS AND DISCUSSION

Physico chemical standardization

Physico-chemical data such as pH, Loss on drying at 105°C, Total ash, Acid insoluble ash, Extractive values were calculated and given in Table 1. Quantitative estimation of active ingredients were also carried out and presented in Table 2. Estimation of biochemical standards were presented in Table 3.

In the present study it was concluded that the physicochemical parameters can be efficiently used for testing identity, purity and strength of the polyherbal formulation (RGM). The data revealed Ash of 7% indicating siliceous matter is not present much and the formulation is comparatively pure. More extractive values of water and alcohol depicted the strength of bioactive molecules present in the formulation.

Table 1: Physico chemical analysis

S. No	Tests	As per analysis
1.	Description	Dark brown colour semisolid drug
2.	**pH**	
3.	Loss on Drying at 105°C	14.1899 %
4.	Total Ash	7.1269 %
5.	Acid Insoluble Ash	0.7476 %
6.	Alcohol soluble Extractive	26.5258 %
7.	Water Soluble Extractive	56.6698 %
8.	Organic Carbon	1.28%
9.	Total Nitrogen	0.82%
10.	Total phosphorous	0.24%
11.	Total Potassium	3.69%
12.	Total Sodium	1.40%
13.	Total Calcium	8.64%
14.	Total Magnesium	3.15%
15.	Total Sulphur	1.20%
16.	Total Zinc	0.56ppm
17.	Total copper	0.03ppm
18.	Total Iron	186.32ppm
19.	Total Manganese	4.22ppm
20.	Total Boron	0.01ppm
21.	Total Molybdenum	0.02ppm

Table 2: Quantitative estimation of active Ingredients

S. No	Phytoconstituents	Yield
1	Total fatty Matter	10.4250 %
2	Alkaloids	2.36 mg/kg
3	Flavanoids	5.63 mg/kg
4	Terpenoids	0.22 mg/kg
5	Saponins	0.13 mg/kg

Estimation of active ingredients

From the above Table 2, Total fatty matter was found to be in higher percentage which was confirmed through GC-MS analysis. Presence of 5.63mg/kg of Flavanoids in the formulation suggested antioxidant potentials of the test drug as flavones are proved antioxidants (6, 7, 8). The antioxidant potential of this formulation was also reported in the *in-vivo* experiments (9).

Estimation of biochemical Standards

Table 3: Quantitative estimation of biochemical standards

S. No	Biochemical Standards	Yield(mg/kg)
1	Total Carbohydrates	0.25
2	Total Protein	0.09
3	Total Fats	0.01

Analysis of Total Microbial count

Table 4: Total Microbial Count

S. No	Bacterial Name	Cells in Sample/g	WHO Limits	Inference
1.	*E.coli*	Nil	10^2	Within Limits
2.	*Salmonella spp.*	Nil	Absent	Absent
3.	*Shigella spp.*	Nil	Absent	Absent
4.	*Enterobacteriaceae*	Nil	10^4	Within Limits
5.	*Pseudomonas aeruginosa*	Nil	Absent	Absent
6.	*S .aureus*	Nil	Absent	Absent
7.	Total viable aerobic count	19x 10^4	10^7	Within Limits
8.	Total fungal count	2 x 10^2	10^4	Within Limits

HPTLC analysis for Alkaloids and Flavanoids:

The Photo documentation of methanol extract of the sample under UV chamber at 254nm and 366nm were presented (Fig1&2)

Fig. 1: HPTLC Profile for alkaloids. 1. Sample solution (5µL), 2.Sample solution (10 µL)

Fig. 2: HPTLC Profile for flavanoids 1.Standard Quercetin (5µL), 2.Sample solution (5µL) 3.Sample solution (20µL)

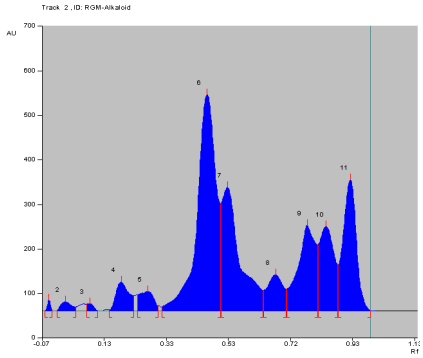

Fig 3: HPTLC chromatogram for RGM (Alkaloids)

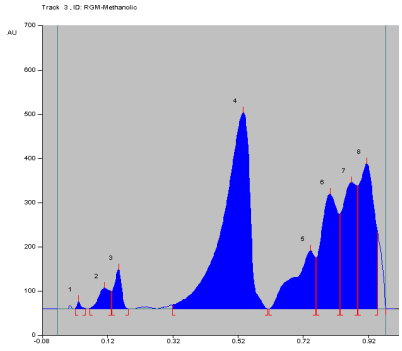

Fig 4: HPTLC chromatogram for RGM (Flavanoids)

HPTLC details of Rasagandhi mezhugu

Source	No. of spots	R_f of various spots	R_f of major spots
RGM	9	0.08,0.18,0.26,0.46,0.52,0.68,0.78,0.84,0.92	0.46,0.52,0.78,0.92
	7	0.03,0.11,0.15,0.54,0.74,0.80,0.87,0.91	0.54,0.80,0.87,0.91

HPTLC fingerprinting of Alkaloids and Flavanoids were given in fig.1&2. Nine active spots at different R_f values were obtained for alkaloids and seven active spots with different R_f values for Flavanoids. The peak for Quercetin from the sample solution had the same retardation factor as that of standard Quercetin (R_f 0.74) (Fig 2).

GC-MS analysis of RGM

The acetone fraction was subjected to GCMS investigation (Table 3). The chromatogram obtained was presented in Fig 3 and the spectrum of major compounds in Fig 4.

Table 4: GC-MS data of Acetone extract of Rasagandhi Mezhugu

S. No	Peak Name	Retention	Formula	%Peak
1.	2-Propenoic acid, 2-hydroxyethyl ester	3.16	C5H8O3	0.2434
2.	2-Propanone, 1-hydroxy-	3.30	C3H6O2	0.4219
3.	Propanoic acid, 2-oxo-, methyl ester	3.54	C4H6O3	0.7165
4.	Propanoic acid, 2-hydroxy-, ethyl ester	3.88	C5H10O3	0.0402
5.	3-Furanmethanol	3.93	C5H6O2	0.0082
6.	Furfural	4.13	C5H4O2	0.4236
7.	2-Furanmethanol	4.43	C5H6O2	0.3181
8.	2-Propanone, 1-(acetyloxy)-	4.63	C5H8O3	0.1097
9.	Butanoic acid, 2-ethyl-3-oxo-, methyl ester	4.83	C7H12O3	0.7201
10.	1H-Imidazole, 4,5-dihydro-2-methyl-	5.39	C4H8N2	0.0768
11.	1,2-Cyclopentanedione	5.57	C5H6O2	0.2855
12.	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	6.25	C6H8O4	0.0852
13.	Heptanoic acid	6.35	C9H18	0.0539
14.	Acetic acid, octyl ester	7.20	C10H20O2	0.0332
15.	trans--4-Nonene	7.38	C7H14O2	0.0353
16.	1,3-Dioxol-2-one,4,5-dimethyl-	7.67	C5H6O3	0.2127
17.	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	7.93	C6H8O3	0.0439
18.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	8.97	C6H8O4	0.6852
19.	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	10.24	C6H6O3	6.6625
20.	Benzoic acid, 4-methyl-	10.80	C8H8O2	0.2868
21.	Phenol, 2-methyl-5-(1-methylethyl)-	10.96	C10H14O	0.0365
22.	2-Methoxy-4-vinylphenol	11.34	C9H10O2	0.0642
23.	Phenol, 2-methoxy-3-(2-propenyl)-	11.99	C10H12O2	0.0978
24.	n-Decanoic acid	12.09	C10H20O2	0.2863
25.	1,2,3-Benzenetriol	12.51	C6H6O3	0.9286
26.	Phenol, 2-methoxy-4-(1-propenyl)-, (Z)-	13.51	C10H12O2	0.0189
27.	1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)-(Myristicin)	14.67	C11H14O3	0.0595
28.	Dodecanoic acid	15.33	C12H24O2	5.5747
29.	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	15.91	C11H12O3	0.0257
30.	Asarone	16.12	C12H16O3	0.4020
31.	Ar-tumerone	16.82	C15H20O	0.1417
32.	Asarone	16.99	C12H16O3	0.1720
33.	Apiol	17.08	C16H32O2	0.0371
34.	2',3',4' Trimethoxyacetophenone	17.25	C11H14O4	0.0323
35.	Tetradecanoic acid, 12-methyl-, methyl ester	17.50	C12H14O4	0.0904
36.	Tetradecanoic acid	18.20	C11H6O3	6.2198
37.	2H-Furo[2,3-H]-1-benzopyran-2-one	18.57	C11H6O3	0.1593
38.	2H-Furo[2,3-H]-1-benzopyran-2-one(Angecin)	19.28	C14H28O2	0.2078
39.	Pentadecanoic acid	19.40	C12H22	0.0492

40.	Phthalic acid, butyl undecyl ester	19.58	C23H36O4	0.0360
41.	Naphthalene, decahydro-1,1-dimethyl-	19.84	C15H30O2	0.0236
42.	1,3-Bis(cinnamoyloxymethyl)adamantane	20.08	C30H32O4	0.4862
43.	Palmitic acid, methyl ester	20.17	C17H34O2	0.1650
44.	Hexadecenoic acid, Z-11-	20.50	C16H30O2	1.6041
45.	Palmitic acid	20.08	C19H34O2	17.1877
46.	4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z)-	21.42	C23H34O2	0.2434
47.	Linoleic acid, methyl ester	22.26	C16H32O2	0.2622
48.	Oleic acid, methyl ester	22.31	C18H32O2	0.3486
49.	Phenol, 4-(3,7-dimethyl-3-ethenylocta-1,6-dienyl)-	22.62	C18H24O	0.9187
50.	Linoleic acid	23.09	C19H36O2	46.1498
51.	(E)-13-Docosenoic acid	23.93	C27H38O8	0.0893
52.	2-Cyclohexyl-2,5-cyclohexadiene-1,4-dione, 4-oxime	24.63	C12H15NO2	0.0340
53.	12-O-Acetylingol 8-tiglate	24.71	C22H42O2	0.0414
54.	1H-Imidazole, 4,5-dihydro-2-(phenylmethyl)-	24.77	C14H22O	0.0991
55.	9,12-Octadecadienoyl chloride, (Z,Z)-	26.07	C18H31ClO	0.0704
56.	(1As-(1aà,4bá,8as)-4a,8,8-trimethyloctahydrocyclopropa(d)naphthalen-2(3H)-one	30.05	C10H12N2	0.0790
57.	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	30.66	C17H19NO3	0.1796
58.	Phenol, 2-methoxy-4-propyl-	31.39	C10H14O2	0.1121
59.	Heneicosane	32.08	C21H44	0.0609
60.	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	32.64	C30H50	0.2592
61.	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-	32.92	C23H32O	0.0312
62.	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	33.37	C11H14O3	0.0652
63.	Cholesta-3,5-diene	33.73	C27H44	0.0963
64.	Piperine	34.04	C17H19NO3	0.7098
65.	N1-Tetrahydrofuran-2-ylmethyl-2-(3,4,5-trimethoxybenzylidene)hydrazine-1-	34.75	C16H23N3O4S	0.1489
66.	3-Isopropyl-6a,10b-dimethyl-8-(2-oxo-2-phenyl-ethyl)-dodecahydro-benzo[f]chromen-	35.05	C26H36O3	0.2165
67.	Cholest-5-en-3-ol (3á)-	36.41	C27H46O	3.4473
68.	2-[3-(1-Hydroxy-1-methyl-ethyl)-6a,10b-dimethyl-7-methylene-dodecahydro-	36.64	C27H38O3	0.2166
69.	2,6-Bis(3,4-methylenedioxyphenyl)-3,7-dioxabicyclo(3.3.0)octane	36.97	C20H18O6	0.4637

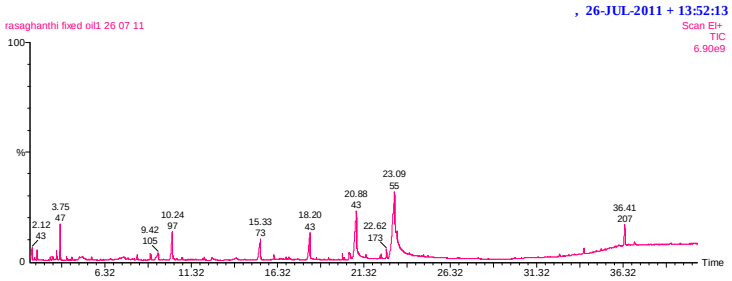

Fig. 3: chromatogram of acetone extract

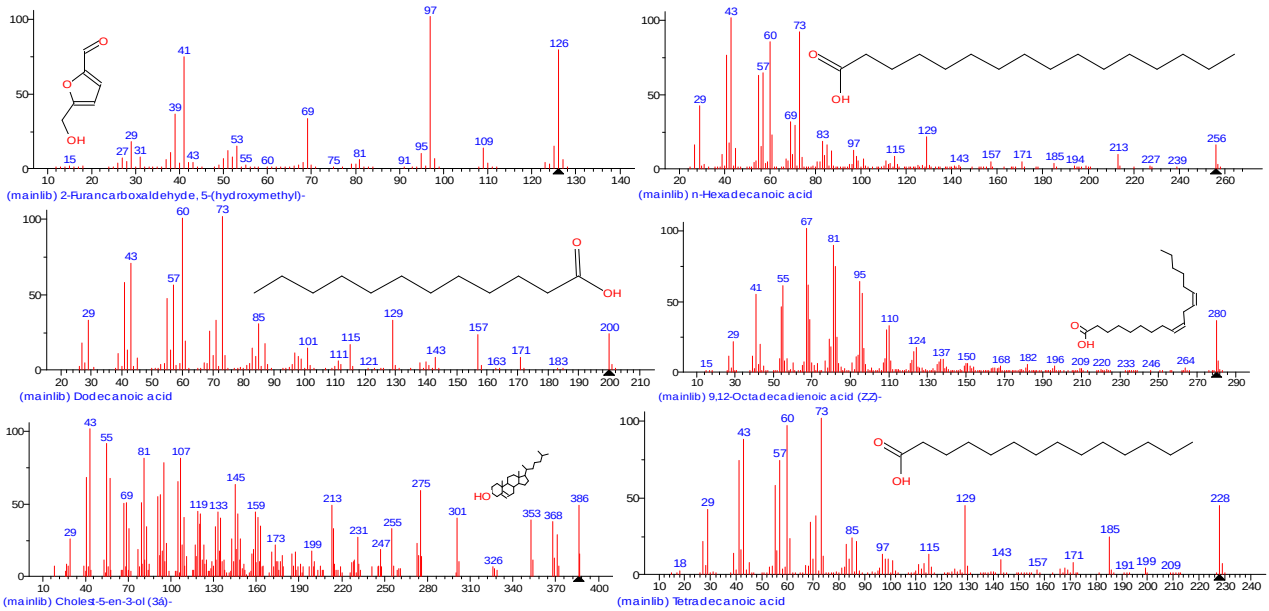

Fig. 4: Mass Spectrum of major compounds

GCMS data presented in Table 3 shows the presence of seventy one bioactive ingredients. The content of fatty acids was higher when compared to other active ingredients. It was found to be 72.8754%. The phenolic content was found to be 1.5607%. The sterols were found to be 3.4473%. Fragmentation pattern of Alkaloids, Coumarins and some aromatic compounds were also present.

CONCLUSION

To conclude the following chemical standards were determined for the Rasagandhi mezhugu.

Test for Identity, Purity and Strength:

Foreign matter- NMT 2%

Total ash- NMT 7%

Acid-Insoluble ash- NMT 0.7%

Alcohol soluble extractive- NMT 27%

Water soluble extractive- NMT 57%

Fatty acids, Alkaloids and Flavanoids are the important constituents present in the sample Rasagandhi mezhugu.

HPTLC profile: HPTLC of methanol extract on Aluminium plate precoated with silica gel60 F_{254}(Merck, 0.2mm thickness) using Toluene: Ethyl acetate: Diethyl ether (60:30:10 v/v/v) as mobile phase for alkaloids show under 366nm,nine spots appear at R_f 0.08 (Blue),0.18 (Red),0.26 (Blue),0.46 (Green), 0.52 (blue),0.68 (Green),0.78(Blue),0.84 (Blue),0.92 (Green) and Toluene: Ethyl acetate: Acetic acid: Methanol (2.5:7.0:0.25:0.25 v/v/v/v) as mobile phase for Flavanoids shown under 280nm, seven spots appear at R_f 0.03(Blue), 0.11(Green), 0.15(Dark blue), 0.54(Light Green), 0.74(Blue), 0.80 (Yellow), 0.87 (Blue), 0.91(Blue).

Total microbial count was within the limits. Heavy metal content may be high if not prepared and processed properly with the specified herbs. It is mentioned in the siddha text that proper treatment with herbs is essential to remove the toxic materials and to enhance the therapeutic potentials. In this formulation presence of Fatty acids and Flavanoids like Quercetin might be performing this action of removing the impurities and reducing the toxicity and enhancing the therapeutic potentials, rendering the formulation to be more human compatible. Further Scientific Validation in these aspects can not only take our traditional metallic preparation to greater strides but also towards their International recognition and acceptance.

ACKNOWLEDGEMENT

We place on record our deep sense of gratitude to our Honourable Vice chancellor, SASTRA University for providing necessary infrastructure and our Registrar for his constant encouragement and motivation. We thank Indian Council of Medical Research (ICMR), New Delhi for funding (No. 61/6-3/2007-HIV/DMS/TRM Dt. 29.05.08).

REFERENCES

1. Deivanayagam CN. Siddha medicines are effective in controlling HIV/AIDS- A report. Proc. National Seminar on the use of traditional medicines in HIV infection and AIDS. Thrissur, Kerala, India ,2002.
2. Ramachandran SP, Pulipani vaidhiyam-500, Thamarai Library, Chennai, 1999, 95, p.72.
3. Agarwal A, Murali B, Quality Assessment of Selected Medicinal Plants, Volume 1, p. 193-196.
4. Quality Standards of Indian Medicinal Plants- Volume 3, 2005: p. 192.
5. Quality Standards of Indian Medicinal Plants- Volume 1, 2003: p. 120-122.
6. Pietta, PG., Flavanoids as antioxidants, J Nat Prod., 2000, 63, p.1035-1042.
7. Bernardi APM., Alarcon CL.et.al; Antioxoidant activity of Flavanoids isolated from Hypericum ternum; J.Chil. Chem. Soc, 52, 2007, p. 1326-1329.
8. Pourmorad F, Hosseinimehr SJ, Shahabimajd N., Antioxidant activity, phenol and Flavanoid contents of some selected Iranian medicinal plants; Afr. J. of Biotech. Volume 5, 11, 2006, p. 1142-1145.
9. Sivasubramanian R., Rama K, Brindha P; Antioxidant property of Rasagandhi mezhugu on paracetamol induced oxidative stress in Albino rats, Indian drugs, 2007, 44, p. 831-840.