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Comparative evaluation and standardization of marketed herbal antiobesity formulation

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ABSTRACT

The present study involved comparative standardization and authentification of medicinal plants and marketed herbal antiobesity formulations. Marketed herbal tablet formulation contains Vrikshamla Ghana and Triphala Ghana main ingredient. The herbal tablet formulation effective in weight loss i.e. Antiobesity effect. Formulations were standardized as per WHO, ICH guidelines and modern scientific quality control procedures. Standardization of tablet formulation was performed by physicochemical parameters, Quality control tests of tablet, IR, TLC, HPTLC. The amount of total phenols were analysed by using Folin-Ciocalteau assay. The amounts of total Flavonoid were analysed using aluminium chloride calorimetricor assay. Free radical scavenging activity was evaluated using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radical. HPTLC chromatogram confirmed quantitative presence of active ingredient in herbal formulation. Total phenol was found to be 18.35mg/tablet. Total Flavonoid was found to be 1.333mg/tablet. The antioxidant activity was found to be 72%.The present standardization study reveals compliance with all the above selected parameters. The application of present study may play significant role in deciding the identity, purity, quality of the herbal tablet formulation and also for fixing standards for this Ayurvedic formulation.

Key words: Herbal formulation, Standardization, HPTLC, IR.

INTRODUCTION

Ayurveda is an intricate but important medicinal system that originated in India thousands of years ago. The term “Ayurveda” thus means ‘the knowledge of life’ or ‘the science of life’ [1].Medicinal plants, for several centuries, have been widely used as a primary source of prevention and control of livestock diseases. Medicinal plants are an integral component of research developments in the pharmaceutical industry [2].The World health organization (WHO) had given a brief protocol for standardization of herbal drugs. Standardization is very prominent to ensure that each finished product that enter market free of adulteration [3].Nowadays there is need to standardize Ayurvedic formulation in uniform quality [4].The purpose of this work was to standardize a marketed herbal tablet formulation for quality and efficacy. Standardization of herbal formulation means confirmation of its identity and determination of its quality and purity [5].This herbal tablet formulation popularly used containing herbs namely Vrikshamla Ghana (*Garcinia indica*), Triphala Ghana (*Three myrobalons*), Shuddha Guggul (*Commiphora mukul*).This formulation widely used for weight loss i.e. Antiobesity effect [6], [7].

MATERIALS AND METHODS

Instrumentation
Digital pH meter (Pico, Lab India) were used, Dissolution test apparatus (Electro lab- USP TDT-08L), Disintegration test apparatus (Electro lab ED-2SAPO), Friabilator(Electro lab – USP EF-1W),Monsanto hardness tester(Dolphin), UV-visible spectrophotometer (Double beam) Shimadzu 1650 PC were used, Fourier Transform Infrared spectrophotometer (model no.84000S SHIMADZU),HPTLC –CAMAG Linomet.

Materials
All chemical used were of analytical grade. Required plant medicine for standardization of herbal formulation was collected form authorized raw drug supplier. This raw drug identifies and authenticate in lab.

1. Collection and Authentification of drug
Dried Kokum and Three myrobalons are collected local market of Kopargaon, Dist-Ahmednagar, India. Its authentification was carried out at botany department, S.S.G.M. College Kopargaon, Dist- Ahmednagar, India.

2. Physicochemical standards for polyherbal formulation
pH
1 tablet was dissolved in distilled water and pH measured by digital pH meter. The calibration of pH meter was done by using solution of 4, 7, and 9.2 respectively [6], [7], [8], [9], [10].

Determination of Total ash
Take 2 gm of sample was accurately weighed in a tarred silica dish at a temperature not exceeding 450^0C until it was free from carbon. Then it was cooled and weighed. The percentage ash was calculated by taking in account the difference of empty weight of crucible and that of crucible with total ash [6-10].

Determination of Acid insoluble ash
The crucible containing total ash was boiled for 5 minutes with 25 ml of dilute hydrochloric acid and covered with a watch glass. The insoluble matter was collected on an ash less filter paper and washed with hot water until filtrate was neutral. The insoluble matter containing filter paper was transferred to crucible, dried on hot plate and ignited to a constant weight. The content of acid insoluble ash was calculated as a percentage acid insoluble ash [6], [7], [8], [9], [10].

Determination of Water-soluble ash
The total ash obtained, boiled for 5 min. with 25ml of water. The insoluble matter was collected on Gooch crucible or an ash-less filter paper and wash with hot water. The insoluble ash was transferred into a tarred silica crucible and ignited for 15 minutes at a temperature not exceeding 450^0C.The total weight of insoluble matter should be subtracted from the weight of the ash. The difference in weights represents the water-soluble ash. The percentage of water soluble ash was calculated [6-10].

Determination of Water-soluble extractive
25 gm of sample were powdered and macerated with 50 ml of water in an enclosed flask for 24 hours. Flask were shaken frequently during first 6 hours and allowed to stand for next 18 hours. After 24 hours filtration was done rapidly. Solvent evaporated in vacuumed evaporator under reduced pressure and temperature. From the weight of dried residue water soluble extractives were calculated [6-10].

Determination of Alcohol-soluble extractive
25 gm of sample were powdered and macerated with 50 ml of ethanol in an enclosed flask for 24 hours. Flask were shaken frequently during first 6 hours and allowed to stand for next 18 hours. After 24 hours filtration was done rapidly. Solvent evaporated in vacuumed evaporator under reduced pressure and temperature. From the weight of dried residue ethanol soluble extractive were calculated [6], [7], [8], [9], [10].

Quality control test of tablet as per IP (2010)
1. Hardness: 5 tablet of sample were taken to measure hardness. The hardness calculated and compared to IP standard.

2. Friability: 20 tablet of sample were used for test. Friabilator was run for 4 min at 25 rpm and percentage friability was calculated and compared with IP standard.
3. Disintegration Test: Disintegration test was performed using 6 tablet of sample. The media used for this test was distilled water and time required to disintegrate the tablet were recorded.
4. Weight Variation Test: 20 tablets were weighed individually. Average weight and % weight variation was calculated and compared with IP limit.
5. Dissolution study: Dissolution study of tablet was carried out using phosphate buffer 6.8 as a dissolution media. The samples were withdrawn for 8 hours at the interval of 45 min. The absorbance of sample measured on UV spectrophotometer and percentage release was calculated [8], [9], [10].

Total Phenolic Content (TPC)
The amount of total phenolic content in polyherbal tablet formulation was determined with Folin Ciocalteau assay with some modifications. In 50µl of sample, add 0.25 ml folin ciocaltea reagent, 2ml of Na_2CO_3 (7.5%) respectively and incubated at 45^0c for 15 min. Absorbance of sample was measured at 765nm using UV-Vis-Spectrophotometer. The calibration curve was plotted as concentration Vs absorbance using Gallic acid as standard. Prepared the dilutions of formulations in the 20,40,60,80 & 100mg/l and results were expressed as milligrams of Gallic Acid Equivalents (GAE) per 100ml of sample (mg GAE/100ml) [11],[12].

Total Flavonoid Content (TFC)
Aluminium chloride colorimetric assay was most desirable analytical method for determination of TFC. In test tube,1ml sample was diluted with 4ml distilled water,0.3ml of 5% $NaNO_2$ and 0.3ml of 10% $AlCl_3$ after 5 minute.2ml 1M NaOH was added and make up final volume 10ml with DW. Tremble the total content of test tube and record the absorbance at 510nm using UV-Vis-Spectrophotometer. The calibration curve was plotted using (+) – catechine (10mg/100ml) as standard. Prepared the dilutions (20, 40, 60, 80 &100mg/1) and result were plotted as milligrams of (+) – catechin equivalents (CE) per 100ml of sample (mg CE/100ml) [11], [12].

***In-vitro* Antioxidant activity**
In-vitro antioxidant potential of sample was determined using DPPH radical scavenging assay by using DPPH radical scavenging assay by using UV-vis-spectrophotometer. Antioxidants react with 2,2-diphenyl-2-picryl-hydrazyl (DPPH) and converts them in to colorless 2,2-diphenyl-1-hydrazine,reporting a decreased in absorbance which measured at 517nm, more rapidly the absorbance reduces, more antioxidant activity of the formulation.3ml of methanolic DPPH solution (24mg in 100ml methanol),was mixed with 10-100µg/ml of sample. Control was also prepared without sample. Reaction mixture was incubated at room temperature for 30min.Decreased in absorbance was read at 517nm and use above formula for calculation of percentage inhibition [11], [12].

$$\%\ inhibition = Ac - As\ /Ac \times 100$$

Ac - absorbance of the control
As – absorbance of the sample

FT-IR Analysis
The IR spectra were recorded using Fourier Transform Infrared spectrophotometer (model no.84000S SHIMADZU) by KBr press pellet technique. KBr was purchased from Merck chemicals, India & was AR grade.

HPTLC Analysis
HPTLC study of marketed tablet formulation was carried out along with the marker compound corresponding to the active ingredient to ensure the presence of active ingredient in the formulation.

Selection of plate and adsorbent
Pre coated aluminum plates with Silica gel 60 F_{254} (10 × 10cm), was used for detection.

Sample preparation
Powder of tablet formulation was dissolved in methanol and shake well. Filter it through whatman filter paper no. 41.Take the filtrate for TLC/HPTLC profiling. Same procedure for standard Gallic acid.

Process

The chromatograph was performed by spotting sample and standards on thin layer of adsorbent, using camag Linomat 5 applicator and developed by using reported Benzene : Ethyl acetate : Formic acid (5 : 7 : 1.5) mobile phase for 30 min. scanning was performed on camage TLC scanner at 254nm wavelength. R_f value of sample compared with R_f value of standard Gallic acid [13], [14].

RESULTS AND DISCUSSION

Table-1: Physicochemical standards for polyherbal formulation

Sr. No.	Parameter	Result
1.	p^H	6.8
2.	Total ash (%w/w)	5.4
3.	Acid insoluble ash (% w/w)	2.24
4.	Water- soluble ash (%w/w)	2.4
5.	Water soluble - extractive value (%)	10.5
6.	Alcohol soluble - extractive value (%)	13
7.	Hardness (kg/cm^2)	5
8.	Friability (%)	0.3
9.	Disintegration time (min)	15
10.	Weight variation (gm)	0.57-0.61

Dissolution study

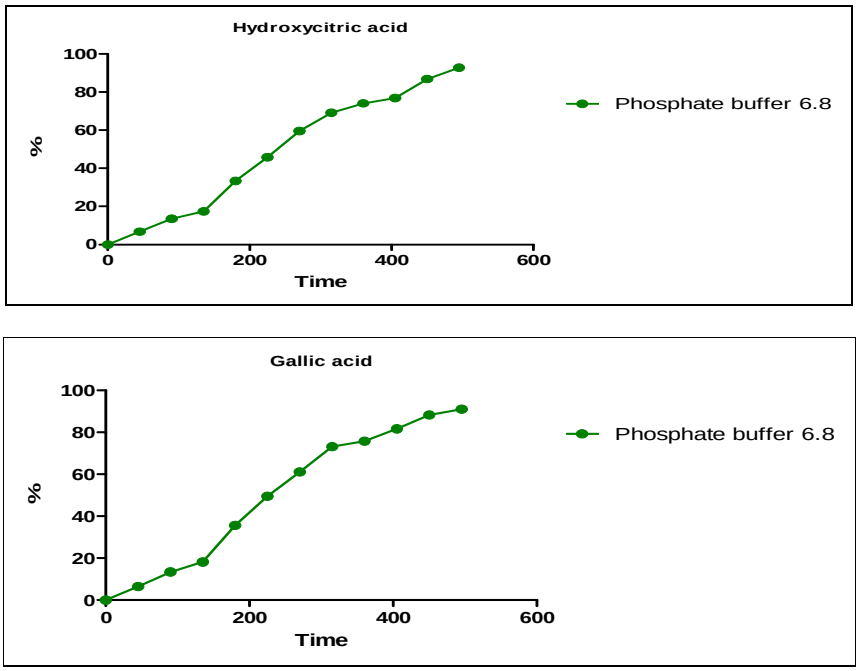

Figure-1: Percentage drug release from tablet

Dissolution study was done using dissolution media phosphate buffer pH 6.8, Gallic acid from three myrobalons and Hydroxycitric acid from Vrikshamla Ghana as active constituent, according to measure % release process of formulation by using UV method. Dissolution study was concluding that, phosphate buffer 6.8 were best media for drug release.

Total Phenolic Content (TPC)

Total phenolic content was determined by using Folin-ciocalteu method. Phenolic compounds exhibiting antioxidant activity by inactivating lipid free radicals, or by preventing the decomposition of hydro peroxides into free radicals [15], [16], [17]. TPC is one of the important estimation since it is related with antioxidant potential of the

formulation. The phenolic content was calculated in terms of Gallic acid equivalent and was found to be 18.35 mg/ tablet. The phenolic compounds responsible for the antioxidant activity.

Total Flavonoid Content (TFC)

Total Flavonoid content is equivalent to catechin. Flavonoids have been shown to be highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various free radicals implicated in several diseases [18],[19].Total Flavonoid content in the tablet was calculated as catechin equivalent and found to be 1.333 mg/tab.

***In-vitro* Antioxidant activity**

In-vitro antioxidant activity of tablet formulation was done by DPPH radical scavenging activity. The method is based on scavenging of DPPH through the addition of a radical species or antioxidant that decolorizes the DPPH solution. The degree of colour change is proportional to the concentration and potency of the antioxidants. A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound under test [19], [20].The tablet showed 72% DPPH radical scavenging activity at 100 ppm concentration. The IC_{50}% value was found to be 6ppm for ascorbic acid and 52 ppm for tablet.

FT-IR Analysis The IR spectrum of formulation, Gallic acid, Hydroxycitric acid was recorded & shown below.

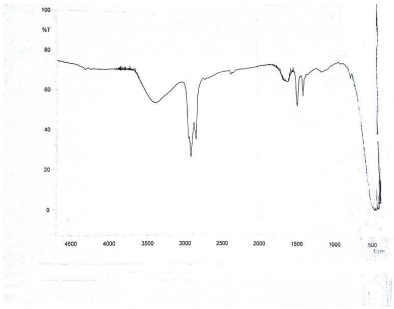

Figure-2: IR spectrum of formulation

Sr. No.	Frequency	Observation
1.	3217cm^{-1},3305cm^{-1}, 3362cm^{-1}	O-H
2.	2952cm^{-1}	C-H
3.	1700cm^{-1}	COOH
4.	1648cm^{-1}	C=C
5.	843cm^{-1}	Out of plan bending for benzene ring
6.	1750cm^{-1}	RCOOR’

Table -2: Interpretation of IR spectra of formulation

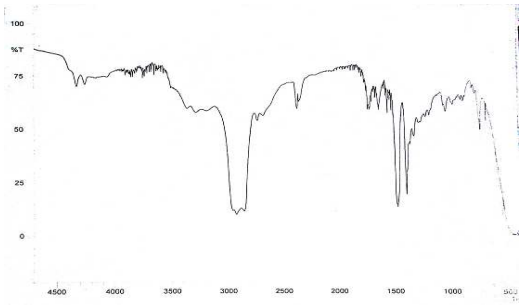

Figure -3: IR spectrum of Gallic acid

Sr. No.	Frequency	Observation
1.	$3272cm^{-1}$,$3335cm^{-1}$, $3493cm^{-1}$	O-H
2.	$2922cm^{-1}$	C-H
3.	$1700cm^{-1}$,$1725cm^{-1}$	COOH
4.	$1662cm^{-1}$	C=C
5.	$852cm^{-1}$	Out of plan bending for benzene ring

Table-3: Interpretation of IR spectra of Gallic acid

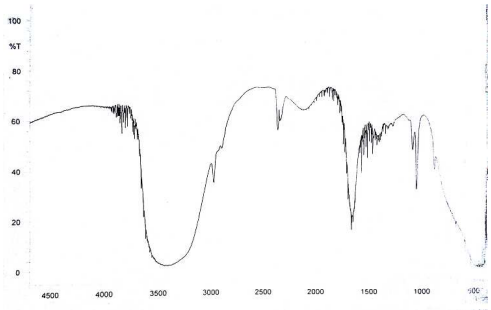

Figure-4: IR spectrum of Hydroxycitric acid

Sr. No.	Frequency	Observation
1.	$3267cm^{-1}$,$3322cm^{-1}$, $3471cm^{-1}$	O-H
2.	$2980cm^{-1}$	C-H
3.	$1791cm^{-1}$	COOH
4.	$1617cm^{-1}$,$1647cm^{-1}$	C=C
5.	$1750cm^{-1}$	RCOOR’

Table -4: Interpretation of IR spectra of HCA

Fig 2.IR spectra of formulation showed the absorption peak of O-H,C-H,COO,C=C,C_6H_6,RCOOR’ and indicating the presence of Gallic acid, Hydroxycitric acid. Fig 3.IR spectra showed the absorption peak of O-H, C-H, COOH, C=C,C_6H_6 and indicating the presence of Gallic acid. Fig 4.IR spectra showed the absorption peak of O-H,C-H,COOH,C=C,RCOOR’ and indicating the presence of Hydroxycitric acid[21].

HPTLC Analysis

The well resolved TLC profile of the marker compound corresponding to the ingredient of tablet formulation was presented in (fig no.5) to authenticate the presence of this ingredient in tablet formulation. Fig. shows that, alternate spot given formulation and standard marker compound (Gallic acid), from right hand side.

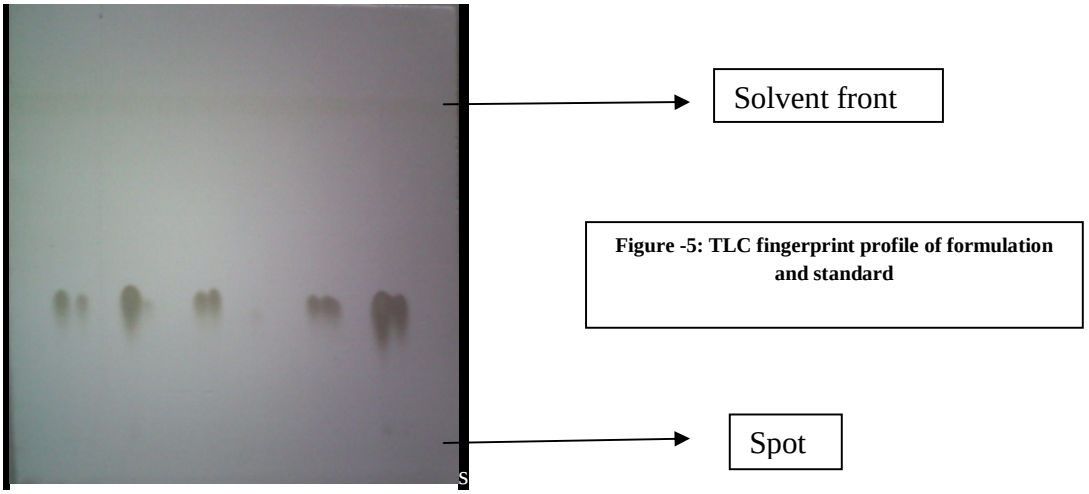

Figure -5: TLC fingerprint profile of formulation and standard

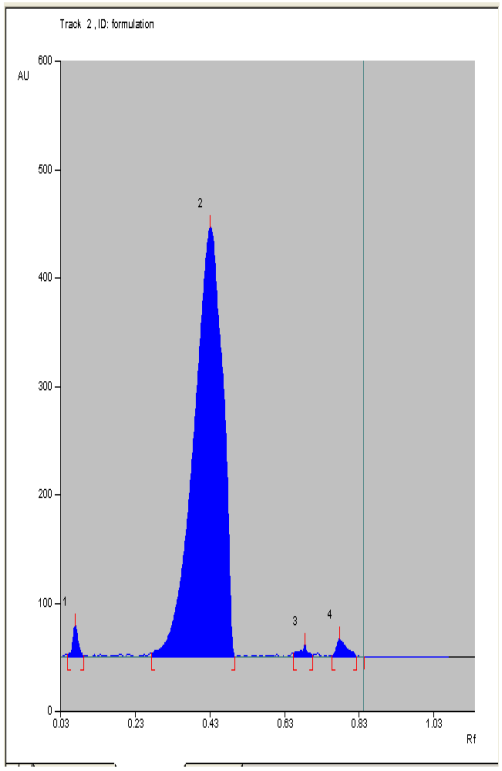

Figure-6: HPTLC chromatogram of tablet formulation

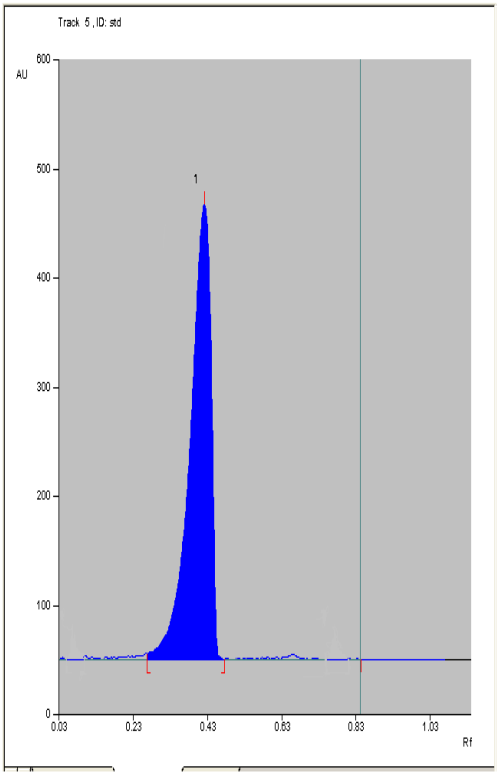

Figure-7: HPTLC chromatogram of standard Gallic acid

The R_f value tablet formulation was found to be 0.43.The R_f value of standard Gallic acid was found to be 0.42.Both R_f value very close to each other. Hence proof that, prominent presence of Gallic acid in tablet formulation.

CONCLUSION

After analysis it can be concluded that, the herbal formulation can standardized by modern scientific quality control protocol. This standardization protocol is prominent to maintain or fixing quality standards of herbal formulation. Result of quality control parameters for this herbal formulation was complying with the standards of Indian pharmacopoeia for tablet dosage form. The present study reported that, formulation shows significant Antioxidant activity, which stabilizes free radicals, before they attack target in biological cells. HPTLC chromatogram confirmed quantitative presence of active ingredient in herbal formulation. Chemical structure of formulation was observed by IR spectrum and was confirmed with respect to standard drug. Hence those chemical constituent present in formulation, which are responsible for Antiobesity action. Thus, present standardization study reveals compliance with all the above selected parameters.

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