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Research Article

PHYSICOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF SINGLE HERBAL FORMULATION - CAPSULE, CONTAINING *EMBLICA OFFICINALIS* GAERTN.

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ABSTRACT

Standardization of *Emblica Officinalis* capsule and its raw material have been carried out in the present study. The study includes antimicrobial evaluation along with estimation of its physicochemical parameters such as ash, extractive values and preliminary phytochemical screening. It also includes quantification of some of the active constituents like Tannins, Gallic acid, and Vitamin C. Study shows Vitamin C, Gallic Acid and Tannin in capsule were 6.32%, 9.96% and 24.12% respectively. *Emblica Officinalis* shows good anti-microbial activity against *E.coli, Salmonella* and *S.aureus.* It reveals standardization profile for drug like *Emblica officinalis*, which would be of immense value in botanical identification and authentification of plant drug and may help us in preventing its adulteration.

Keywords: *Emblica officinalis*, Antimicrobial, Tannins, Gallic Acid, Vitamin C

INTRODUCTION

During the past decade, the therapeutic use of herbal medicine is gaining considerable momentum in the world. The use of herbal medicine due to toxicity and side effects of allopathic medicines, has led to sudden increase in the number of herbal drug manufactures. Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since antiquity. The practices continue today because of its biomedical benefits as well as place in cultural beliefs in many parts of world and have made a great contribution towards maintaining human health. Therefore, reproducible standards of each plant are necessary for effective quality control to prevent adulteration.

Standardization is a system to ensure that every packet of medicine that is sold has the correct amount and will induce its therapeutic effect[1,2,3]. Standardization serves number of purposes including: batch to batch consistency, confirmation of correct amount of dosage or extract per dosage unit, positive control to indicate possible loss or degradation during manufacturing.

Emblica officinalis (Amla or Indian gooseberry) is the fruit of this deciduous tree found mainly in India. This plant belongs to the family Euphorbiaceae[4,5,6]. The fruit of this plant is round shaped with vertical stripes. It is greenish yellow in color and tastes sour. The fruit is fibrous in nature and rich in natural vitamin C[7,8,9]. This fruit is used as the main ingredient in the ayurvedic tonic Chyavanprash[10,11]. It is also used along with several other herbs as an ayurvedic tonic. The Indian gooseberry or Amla has cooling, diuretic and laxative properties. It helps in cleansing the mouth, and strengthens teeth and bones. It increases the red blood cell count and helps to promote good health. It also has antioxidant properties[12,13]. Apart from vitamin C, amla also contains cytokine like substances such as zeatin[14,15]. The dried Amla fruit is useful in the treatment of Hemorrhage, diarrhea, and dysentery[16,17]. Amla has laxative properties and therefore useful in the treatment of constipation and piles. It also has antibacterial properties and helps in preventing infections and healing ulcers[18,19]. It also helps in preventing skin infections. Amla has cell rejuvenating properties and therefore used in maintaining good health of skin and hair. It is widely used in preparation of hair shampoos. Amla helps to keep the hair glossy and shining. It also helps to prevent dandruff. Amla is also known to have anti aging properties[20,21,22].

MATERIALS AND METHODS

Preparation of Test Drug

We have taken various trials of different combinations of extract: herb powder ratio for the preparation of the capsule. Depending on the desired activity the composition of the two was finalized to a ratio of 3.1: 1. For 300 capsules of size "0", 84 g extract and 27 g of dried whole fruit powder of *E. officinalis* were mixed in a homogenizer and filled 370mg of the mixture in each capsule.

Physicochemical studies[23, 24, 25,26,27,28]

Physicochemical parameters were determined as per guidelines of WHO. Raw materials were evaluated for total ash value, acid insoluble ash, moisture, alcohol soluble extractive value, water soluble extractive value, pH (1% w/v) and the finished product was evaluated by weight variation, pH (2%w/v), total ash value, acid insoluble ash, moisture, alcohol soluble extractive value, water soluble extractive value, disintegration time, dissolution using standard pharmacopoeia methods. Tannin content was estimated by gravimetric method, Vitamin C by UV Spectrophotometer and Gallic Acid content by High Performance Liquid Chromatography in both raw materials and finished product.

Tannin content: Both raw materials and finished product were analyzed for content of Tannin by Lohra's method[29]. For the determination, accurately weighed sample was taken in respective conical flasks. 100 ml of distilled water was then added and shaken well and kept on a rotary shaker for 6 hours and then overnight. Then filter the solution and this filtrate is used as the test solution. For the titration 75 ml distilled water is taken in a conical flask and 2.5 ml Indigo sulphonic acid is added. 10 ml filtrate is then titrated with 0.1 M Potassium permanganate [30].

Vitamin C content

Accurately weigh the sample and dissolve it in 75 ml of m-Phosphoric acid (m-PA) taken in Stannous chloride ($SnCl_2$) solution and mix well. Filter the solution and the filtrate is used as the sample for further analysis. Ascorbic acid is used as the standard for preparation of standard graph which ranges from 0µg – 250µg concentration (0ng serves as a blank). Make up the volume to 2.5 ml with m-PA. Add 0.5 ml of 2% Dinitrophenyl hydrazine (DNPH). Incubate it for 1 hr at 50°C. Then add 2.5 ml of 85% sulphuric acid and take the reading at 540 nm on a UV Spectrophotometer.

Gallic acid content by High performance Liquid chromatography

HPLC analysis was performed on a Shimadzu LC-20AD pump system equipped with a Shimadzu SPD-20AT UV- Visible detector with the detection wavelength set at 272 nm and 20µL Rheodyne injector loop . A column was a reversed-phase (Luna C18 4.6 mm x 260 mm – particle size 5µ) eluted at a rate of 1.0 mL/min with a solvent system {Water: acetonitrile: Glacial Acetic acid – 9: 1: 0.2 (V/V)}. Sample was prepared in the HPLC grade methanol.

Microbial contamination

For the safe use of the plant drug, microbial count was carried out as per procedure of Indian pharmacopoeia 2007 and WHO Guideline. It included total bacterial count, Total yeast and mould Count, Presence of *Escherichia coli, Salmonella abony, Pseudomonas aeruginosa,* and *Staphylococcus aureus.* Pure culture of *Escherichia coli* (NCIM: 2065; ATCC: 8739), *Salmonella abony* (NCIM: 2257 NCTC: 6017), *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC 6358) obtained from NCIM Pune were used as control. The media used for the microbial limit test were of HiMedia Pvt. Ltd

Heavy metal Analysis

Heavy metal analysis was performed using Shimadzu AA-6300, HVG and digestion was done on CEM MARSExpress microwave digestive system. Sample amount 0.5g and 8ml 69% Nitric acid were taken in the Teflon PFA 75ml vessels. Parameters used for the digestion were Power 400 W with 100%, Ramp 20 minutes to attempt temperature 150^{o}C and hold for 10 minutes. After digestion process completed the sample was diluted up to 50ml by distilled water and filter through whatman filter paper No. 1. The standards of Lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg) were prepared and the calibration curve was developed for each of them. Samples were analyzed by using these standard curves. The permissible limit for Heavy metal in herbal drugs is consider as per describe limit given by Department of Ayush[31].

Antimicrobial Activity [32, 33, 34]

Test Organism

All the clinical strains were procured from NCIM, Pune that included one gram positive organisms and 3 gram negative organisms namely *S.aureus* (ATCC 6358, NCIM 2079), *E.coli* (NCIM NCIM: 2065; ATCC: 8739), *P.aeruginosa* (ATCC 9027, NCIM 2200) and *Salmonella* (NCIM 2257, NCTC 6017). Immediately they were sub-cultured by inoculating a loopful in Nutrient Broth (HiMedia, M002) and then incubated at 35-37°C for 18-24 hours. They were then streaked onto Nutrient agar (Hi Media, MM 012) plates and the plates were inverted and incubated at 35- 37°C for 18-24 hours. They were then stored at 4°C till use.

In Vitro Anti Bacterial Study

Muller Hinton Agar (3.7%) was mixed with hot distilled water and autoclaved at 15 lb pressure for 15 minutes. After autoclaving allow the media to cool to about 45°C before use. Then the medium was poured into sterilized Petri dishes with a uniform depth of approximately 4 mm. The agar medium was allowed to solidify and then stored at 4°C till used for further analysis.

Sample Preparation

The contents of 1 capsule (370 mg) were suspended in 5 ml of sterile distilled water aseptically. In a simillar manner sample was prepared using 2 capsules (740mg). Both these samples were used for analysis.

Cup Plate Method

The cup plate method was done for each isolate on Mueller-Hinton agar. The turbidity of the broth was adjusted according to 0.5 Mc Farland standards (NCCLS 1993) by adding sterile saline. A sterile cotton swab was saturated by dipping into standardized bacterial culture. Lawn culture of the test strain was prepared by swabbing to give uniform inoculums to the entire surface. The plates were allowed to dry, after which wells were bored in the middle of the well with the help of a cork borer and 0.1ml of the sample was loaded into the well. The plates were first incubated at 25° C for 30 minutes and then shifted to 37°C for 18 – 24 hours. After incubation the plates were examined and zone of inhibition were measured. All the tests were carried out in duplicates. To screen the anti bacterial activity against the tested organisms a standard was used which was antibiotic Amoxycillin (5 mg/ml) which showed a good zone of inhibition against the tested organism[35].

RESULTS AND DISCUSSION

Physiochemical studies

The quantitative determination of some physicochemical parameters (Table No.1) is useful for setting standards for drugs. The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The moisture content of the drug is not too high, thus it could discourage bacteria, fungi or yeast growth. Equally important in the evaluation of crude drugs, is the ash value and acid insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica. Extractive values help us in determining the amount of active constituents and is done on plant materials for which as yet no suitable chemical or biological assay exists. The phytoconstituents quantified in the present study exhibit great deal of medicinal importance like Vitamin C and Gallic Acid, it act as an anti-oxidant and immunomodulatory agent. Present study shows Vitamin C content in Extract, powder, capsule were 7.12%, 0.70% and 6.32% respectively (Table No. 1), while Gallic Acid content in Extract, powder, capsule were 11.96 %, 6.75% and 9.96% respectively (Table No. 1, Fig. 1). The quantified values of the above phytoconstituents can be used as a major tool for obtaining a quality control profile for a drug.

Table 1: It shows determination of proximate parameters of *Emblica Officinalis* Fruit extract and capsule

Sr No.	Test	Extract	Powder	Capsule
1	Description	Blackish brown colour powder.	Brown colour powder.	Clear, trans. Hard gelatin capsule containing Blackish Light brown colour powder.
2	Size of capsule	-	-	Zero
3	Wt. of 20 Filled Capsule (g)	-	-	12.131
4	Wt. of 20 Empty capsule (g)	-	-	1.929
5	Wt. of Net Content (g)	-	-	10.202
6	Av. Net Content Per capsule (mg)	-	-	510.10
7	pH	3.04 + 0.02	2.79 + 0.05	3.10 + 0.01
8	Total Ash (%)	4.12 + 0.03	3.32 + 0.03	3.44 + 0.04
9	Acid Insoluble Ash (%)	1.05 + 0.05	0.83 + 0.04	0.94 + 0.02
10	Water-Soluble Extractive (%)	88.50 + 0.03	57.79 + 0.03	73.43 + 0.26
11	Alcohol Soluble Extractive (%)	85.64 + 0.39	48.61 + 0.49	67.14 + 0.02
12	Assay of Tannin by titration (%)	23.09 + 1.00	28.28 + 0.12	24.16 + 0.05
13	Vitamin C by UV (%)	7.14 + 0.02	0.70 + 0.10	6.33 + 0.02
14	Gallic Acid by HPLC (%)	11.79 + 0.21	6.52 + 0.24	9.79 + 0.21
15	Moisture by Karl Ficher (%)	4.29 + 0.07	5.68 + 0.22	1.86 + 0.08
16	Bulk density (g/ml)	0.400 + 0.01	0.420 + 0.01	0.730 + 0.02
17	Disintegration time	-	-	08 min 03 sec
18	Dissolution Test (%)	-	-	71.55 + 0.28

- = Not Applicable; *Data mentioned in Mean + Standard Deviation (Where n=3)

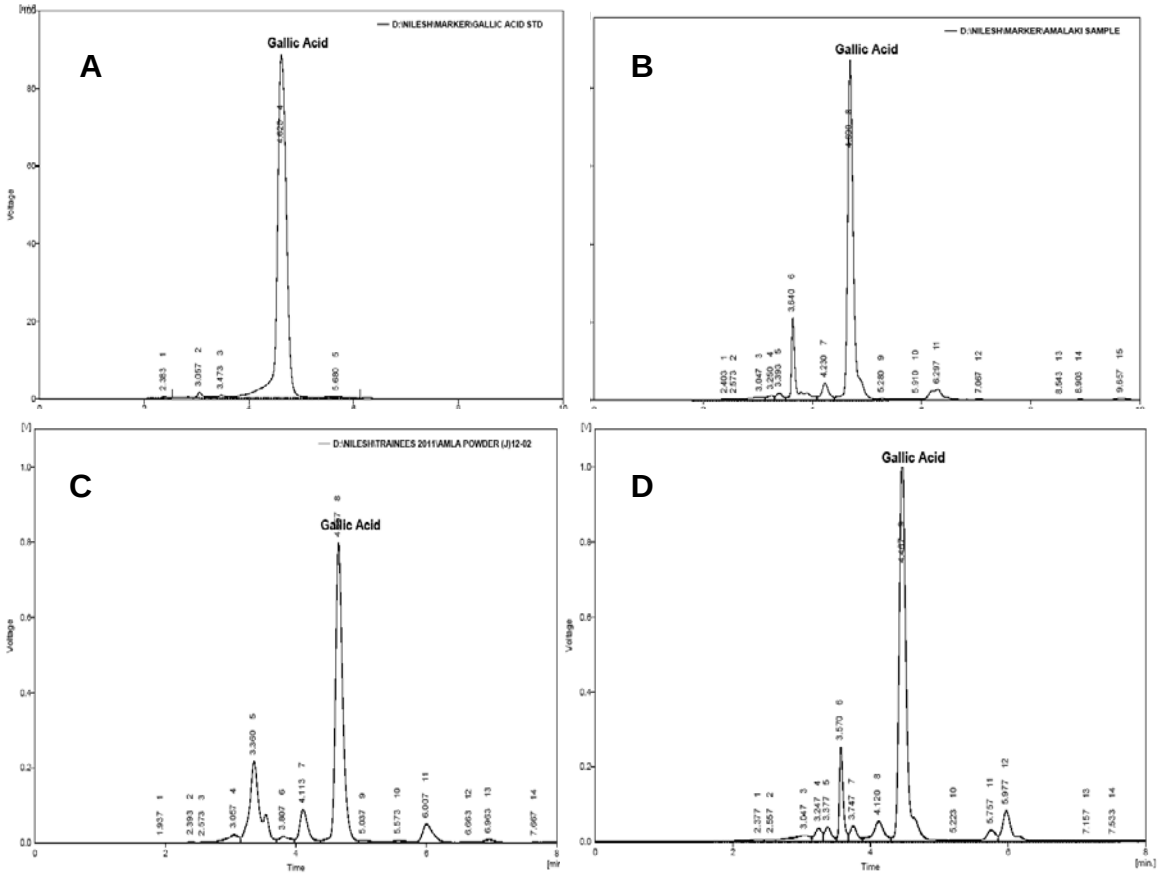

Fig. 1: It shows HPLC Chromatogram, A: Gallic acid Standard, B: *E. Officinalis* Fruit Extract, C: *E. Officinalis* Powder, D: *E. Officinalis* Capsule

Microbial contamination

Microbial analysis study such as Total bacterial count, Total yeast and mould count, *Escherichia coli, Salmonella, Staphylococcus aureus and Pseudomonas aeruginosa* are shown in Table No.2 which indicates that selected capsules passes the limit for microbial contamination. Therefore, the samples investigated were free from microbial contamination.

Heavy metal Analysis

The samples were analyzed for the presence of heavy metals. The results (Table No. 3) showed that mercury was not present in the sample; the presence of lead and Arsenic were found only to be less than 0.5 ppm. Although, there was minor presence of some heavy metals but the sample did not exceed the limit given according to the WHO guidelines. The samples investigated were well below the permissible limits from heavy metal, results are tabulated in Table 3.

Anti-microbial activity

The samples at 74mg/mL and 148mg/mL concentrations were tested for the 4 pathogens. The results are tabulated in Table 4. Antimicrobial activity was seen at both the concentrations. *E. coli* and *Salmonella spp* showed increase in activity with increasing sample concentrations.

Table 2: It shows the Microbial Analysis Report of *Emblica Officinalis*

Sr. No	Name of Pathogen	Amla Extract (cfu/g)	Amla Powder (cfu/g)	Amla Capsule (cfu/g)
1	Total Bacterial Count	2×10^2	7×10^2	4×10^2
2	Total Fungal Count	Absent	Absent	Absent
3	*E.coli*	Absent	Absent	Absent
4	*Salmonella abony*	Absent	Absent	Absent
5	*Pseudomonas aeruginosa*	Absent	Absent	Absent
6	*Staphylococcus aureus*	Absent	Absent	Absent

Table 3: It shows the Determination of Heavy Metals of *Emblica Officinalis*

Sr. No.	Heavy Metals	Amla Extract (ppm)	Amla Powder (ppm)	Amla Capsule (ppm)
1	Pb	0.24 + 0.03	0.88 + 0.02	1.76 + 0.10
2	Cd	0.16 + 0.01	0.03 + 0.01	0.08 + 0.02
3	As	0.12 + 0.03	0.54 + 0.09	0.09 + 0.01
4	Hg	Not Detected	Not Detected	Not Detected

* Data mentioned in Mean + Standard Deviation (Where n=3); Not Detected = Result less than detection limit

Table 4: It shows the mean zone of inhibition exhibited by the test organism against *Emblica Officinalis*

	Amla Capsule (370mg)	
Organisms	**Sample conc. 7.4mg/100µl**	**Sample conc. 14.8mg/100µl**
E.coli	5 mm	13 mm
P.aeruginosa	5 mm	5 mm
S.aureus	6 mm	6 mm
Salmonella spp	5 mm	13 mm

CONCLUSION

The present study is focused on standardization & antimicrobial activity assessment of single herb capsule. Results of physicochemical parameters showed that used raw materials & finished product were good in quality. The anti-oxidant compounds like vitamin C, tannin and gallic acid are good in quantity in the capsule and its raw material. Extraction of *Emblica officinalis* in different solvents were analyzed for anti-microbial activity from that methanolic extract showed good anti-microbial activity against *E.coli, Salmonella* and *S.aureus.* The results of assay, proximate, heavy metal and microbial analysis show that the single herb capsule is standardized for its composition and quality.

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