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PHYTO ANALYSIS AND COMPARATIVE ESTIMATION OF TPC IN EMBLICA OFFICINALIS QUALITY PARAMETERS IN MARKETED AND FOREST SAMPLES.

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ABSTRACT

Emblica officinalis (amla) undeniably a powerhouse of nutrients. It is a very delicious fruit. It is also known as Indian gooseberry. It belongs to phyllanthaceae family. Emblica exhibits strong antioxidant activity. It is one of the most important plants in the traditional ayurvedic medical system as well as in other traditional health systems for immune modulatory, anti-inflammatory, antiulcer, hepatoprotective, and anticancer actions. The increase in demand of medicinal plants for the commercial herbal medicine sector led to the indiscriminate and unscientific collection without any consideration for the quality of the material collected. The ocular observations of the market sample of Aonla fruits indicate that mixing of old and diseased parts of samespecies and other adulterants is rampant in the local market. Laboratory analysis shows that in one kilogram of market sample, more than 20% raw material was found adulterated in all the selected species. Checking and testing the quality of proposed raw herbals traded in the markets and compared to the freshly collected raw material from forest.

KEYWORDS: E. Officinalis, Phyto Analysis, TPC, Marketed samples and Forest Samples.

INTRODUCTION

Indian Systems of Medicine (ISM) predominantly use plant material for the preparation of medicines. But Medicinal plant industry faces the problem of raw material supply and its quality. Adulteration and substitution of non genuine plants is reported to be rampant in near absence of assured supply of genuine medicinal plants as raw material. There is poor law

enforcement system at the field level. In present study to evaluate the quality of medicinal plants namely *Emblica officinalis* are evaluated comparatively for being used as a quality medicinal plants material resourced from local market and natural forest region on the basis of physical and phytochemical parameters. The safety and quality of raw medicinal plant materials and finished products depend on intrinsic (genetic) or external (environment, collection methods, cultivation, harvest, post-harvest care, transport and storage practices) factors. The WHO (2003) guidelines on good agricultural and collection practices (GACP) for medicinal plants are important initiative to ensure good quality of herbal medicines and environmentally sound cultivation practices for sustainable production and utilization of medicinal plants.

MATERIAL AND METHOD

1. **Sample Collection:** The collection of plant material which in general used for medicinal purposes in various forms was done for present study in two ways i.e. collection from forests and collection from traders. The forest regions of Raisen districts of Madhya Pradesh were chosen for the collection of medicinal plants directly from forest for the purpose of study. The most common plant species that are extensively used medicinal plants in India as well as other parts of the world are considered in the present research work with due respect to their high commercial demand as per the objective of present study. The selected plant species is *Emblica officinalis* (Amla).

2. **Morphological and Physical Quality Evaluation**: The quality of raw material and herbal crude drug were assessed on of morphological and physical basis because each and every plant depicts unique characteristics that could be helpful in identifying the purity of raw plant drug or presence of any substituted materials or adulterants.

Morphological Evaluation: This involves the direct ocular analysis of raw material or samples with particular amount of dried material is analyzed using magnifying lens. The identification was done by the help of some taxonomist, consulting flora, Ayurvedic practitioners and experts (Mishra, et al., 2009).

Physical Evaluation: The physical evaluation of plant samples are done for size, colour, texture, shape, odour and taste with reference methods of WHO (1998). The shape of drugs are done with reference methods of Kamboj (2014).

3. **Phytochemical extraction**: The fruits of *E. Officinalis* were collected, cleaned and subjected to shade drying then grounded into a fine powder. Soxhlet extraction with 200 ml of pure distilled water of was separately done using 5 grams of dried powder at 80ºC to obtain fruit aqueous extract respectively; which were then subjected to concentration in hot water bath at boiling temperature till the complete evaporation of solvent and stored in refrigerator.

4. **Preliminary Analysis of phytoextract**:- A small portion of the dry extracts was subjected to the phytochemical test using Harbourne's (1983) methods to test for alkaloids, carbohydrates, glycosides, saponins, phenols, flavonoids, protein and amino acid.

Test for alkaloids: About 0.2 g extract warmed with 2% H_2SO_4 for two minutes, filtered and few drops of Dragendorff's reagent added orange red precipitate indicates the presence of alkaloids.

Test for tannins: Small quantity of extracts mixed with water, heated, filtered and ferric chloride added. A dark green solution indicates the presence of tannins.

Test for terpenoids: About 0.2 g extracts were mixed with 2ml chloroform ($CHCl_3$) and concentrated H_2SO_4 (3ml) was carefully added to form a layer. A reddish brown coloration of the interface formed indicating the presence of terpenoids.

Test for saponins: About 0.2g of the extracts shaken with 5ml of distilled water and then heated to boiling froth (appearance of a creamy mix of small bubbles) shows the presence of saponins.

Test for flavonoids: Extract of about 0.2 g dissolved in diluted NaOH and HCl added. A yellow solution that turns colourless indicates the presence of flavonoids.

Test for glycosides: The extracts hydrolysed with HCl solutions and neutralized with NaOH solutions. A few drops of Fehling solution A and B were added. Red precipitate indicates the presence of a glycoside.

5. Estimation of TPC: The total phenolic content of the extracts was determined using to the Folin-Ciocalteu method (Singleton et al., 1999) with suitable modification. The extracts were suitably diluted with their respective solvents and oxidized with Folin-Ciocalteu reagent, and the reaction was neutralized with sodium carbonate. The absorbance of the resulting blue colour was measured at 750 nm after 60 min. Using gallic acid as standard total phenolic content (standard curve was prepared using concentrations 0-500 mg/L) was expressed as mg GA equivalent/L of extract.

RESULTS AND DISCUSSION

The preliminary phytochemical analysis of these extracts for the presence of alkaloids, carbohydrate, tannins, terpenoids, saponins, flavonoids and glycosides are being depicted in table 1.

Table 1: phytochemical extraction of *E. officinales* (crud plants drug and Market samples).

costituents	*E.officinales*(crud plants drug)	*E.officinales*(Market samples)
Alkaloids	-	+
Carbohydrates	+	-
Glycoside	+	-
Saponis	+	-
Phenols	+	+
Flavonoids	+	+

[(+) means present and (-) means absent]

When compared with gallic acid standard curve (figure 1) according to the spectrophotometric measurements at 750 nm, the absorbance of methanolic extracts of *E. officinales* are being presented in table 2. Estimation of total polyphenolic content in methanolic extracts of *E. officinalis* and comparison with Gallic acid standard curve. Single beam visible range digital microprocessed spectrophotometer from Electronic India model EI-2305 Instrument are Used.

Table 2: Gallic acid as standard concentration Vs absorbance at 765 nm to plot standard curve estimation in samples using Folin-Ceucaltues Method.

S.N.	Concentration (µg/ml)	Absorbance (λ)
1	10	0.237
2	20	0.592
3	30	0.870
4	40	1.159
5	50	1.532

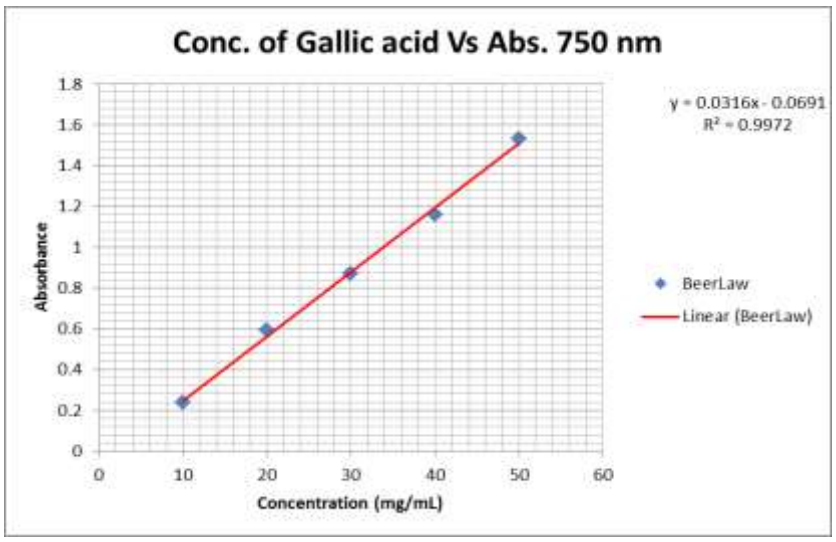

Figure 1: Standard plot for known concentration of Gallic Acid Standard. The Graph is obtained from Excel 2010 linear regression function.

The absorbance of the methanolic phytochemical extracts obtained from the plant materials *E. officinalis* are collected from market prepared in concentration of 100 mg/ml at 765 nm using Folin-Ceucaltues Method to obtain concentration compared to Gallic acid standard plot depicted in table 3.

Table 3.

S.N.	Extracts	Concentration (mg/ml)	Absorbance (λ)	TPC in mg/ml	Percentage TPC
1	*E. officinalis*	100 mg/ml	0.837	28.7	28.7%

The absorbance of the methanolic phytochemical extracts obtained from the plant materials *E. officinalis* are collected from forest prepared in concentration of 100 mg/ml at 765 nm using Folin-Ceucaltues Method to obtain concentration compared to Gallic acid standard plot depicted in table 4.

Table 4.

S.N.	Extracts	Concentration (mg/ml)	Absorbance (λ)	TPC in mg/ml	Percentage TPC
1	*E. officinalis*	100 mg/ml	0.956	32.5	32.5%

Selected parts of *E. officinales* (fruits) collected from natural forest and local market of Bhopal. Due to morphological and physical evaluation it is clear that, maximum proportion of good quality fruits was found in sample collected from natural forest on the contrary very less proportion of good quality fruits was found in sample collected from market. Adulteration is more in the samples collected from market whereas nil the samples collected from forest. The proportion of diseased fruits was more in market sample in contrast to nil in forest samples. From the result the TPC value is higher in control samples (natural forest samples) than the market samples.

CONCLUSIONS

There is a long history of the traditional Indian system of medicine which describes the importance of plants as a potential source of useful structures for the development of new drugs and chemotherapeutic agents (Tona et al., 1998). Substitution or intentional adulteration is not a common practice in the local market. The adulteration was found more intense only during less production of fruits form natural forests and high demand by Ayurvedic industries. More demand and less supply forces stakeholders to adopt malpractices like mixing of similar looking fruits, chaff matter etc. in genuine material (Mishra et al, 2010). Here in the present study Phyto Analysis and Comparative Estimation of TPC in *Emblica Officinalis* Quality Parameters in Marketed and Forest Samples, the forest samples are rich in total phenol content as compare to market samples. Consequently from this study it can be concluded that the natural forest fruit extracts of *E. Officinalis* are rich in TPC the potential of which could be utilized in many ways after further advance studies and proper data generation for the development of new chemotherapeutic agents.

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