In-vitro Anti-Cancer Study of Bio-transformed Material (Extract) of *Embellica officinalis* by MTT Assay

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Abstract:

This work aims to determine the in-vitroanti-cancer activity of biotransformed material (extract) of *Embellica officinalis* fruits. Various concentrations of the biotransformed material (extract) of *Embellica officinalis* were tested. The MTT assay method was used using A549 cell lines, LN Cap-FGC cell linesand MDA-MB cell lines. The result shows promising effect of biotransformed material (extract) of *Embellica officinalis*. The IC_{50} values were found to be less than10 µg against A549 cell lines, the activities against LN Cap-FGC cell lines were found to be 10 µg and IC $_{50}$ values were found to be 10 µg against MDA-MB cell lines

From this study it was evident that the cancer cell growth was significantly inhibited by treatment with biotransformed material (extract). Hence, in vitro studies of *Embellica officinalis* was found to have a potential anti-cancer activity.

Keywords:
Embellica officinalis, MTT assay, A 549 cell lines, LN Cap-FGC cell lines, MDA-MB cell lines, anti-cancer activity.

Introduction:

A proper health care system can be established, supplying low cost medicine to population by using various medicinal plants. Medicinal plants are usually used for Ayurvedic, Unani and other treatments in rural areas. Recent discovery shows that these plants have fewer side effects than the Allopathic medicine. The medicinal importance of these plants is due to the presence of secondary metabolites present in them. Some of the important bioactive compounds are alkaloids, glycosides, triterpenoids, terpenoids flavonoids, polyphenols, reducing sugars, saponins, steroids and tannins etc. Among the plants, *Embellica officinalis* has been used in ayurvedic medicine and has a wide number of traditional uses including internal use for eczema, indigestion. *Embellica* fruits have been considered beneficial in cough, asthma, bronchitis, cephalalgia, ophthalmopathy, dyspepsia, colic, flatulence, hyperacidity, peptic ulcer,erysipelas, skin diseases, leprosy, haematogenesis, inflammationsin reducing acidity, inflammation and wound healing.[1]Cancer is the abnormal growth of cells with uncontrolled division resulting in increased number of cells [2]. In spite of a number of new anti cancer drugs the prevalence of cancer has been found increased now-a-days. Cancer is considered as one of the serious disease which is characterized by abnormal or uncontrolled growth of tissues. It has become a major cause of death and there is an urgent need for its control. The most important drawback of the current cancer therapeutic practices such as chemotherapy and radiation therapy is the suppression of immune system. Since plants have been a prime source of natural products for the treatment of various diseases and they are

also highly effective conventional drugs for the treatment of many forms of cancer [3-5].*Embellica officinalis* belonging to family Phyllanthaceae has been widely used in homeopathic medicine for the treatment of many diseases [6]. It has been reported to possess many pharmacological activities, which include antioxidant[7], anti-inflammatory[8],antibacterial[9],antifungal[10]and antiviral activity[11]. It also possess cytotoxic as well as tumor reducing potential [12]. Literature survey revealed that *Embellica officinalis*, is used in traditional medicine all over the world for its antiseptic property as well as to treat certain skin disorders.

The process in which suitable modification of substrate structure is achieved by biological enzyme catalysis is termed as biotransformation.

There are the following three ways with the help of which the biotransformation process is carried out.

1. With purified culture of microorganisms. Ex. Bacteria or Fungi or Yeast.
2. With plant cell culture or tissue culture
3. With isolated purified microbial or plant or animal cell enzyme.

In the current context, general goals of biotransformation can be listed as follows-

- Specific modification of substrate molecule via selective transformation reaction
  Partial degradation of substrate molecule into desirable metabolites by means of controlled microbial reaction or reaction pathway.
- Extension of substrate or by the use of biosynthetic reactions to form artificial products.

The first processes which were successfully accomplished by biotransformation were-

- The oxidation of alcohol to acetic acid by Bacterium xylinum.
- The oxidation of glucose to gluconic acid by *Acetobactoracetii*.
- Acyloin formation from benzaldehyde and acetaldehyde through yeast.

But, biotransformation gained it's present status only after discovery of microbial transformations of steroids viz. Reduction of and rostensdione to testosterone through yeast, 11-hydroxylation of progesterone by *Rhizopus arrhizus* (Peterson & Murray 1952).In the aftermath, diverse transformations of substrates frommany different groups and types of compounds were found, some of which were usefulin chemosynthesis.

Very few biotransformation studies have been carried out regarding the chemical study of biotransformed products and their pharmacological evaluation. Biotransformation with alcohol and water have been done however, the biotransformation with cow urine has not been done yet. It was therefore envisaged to evaluate the biotransformation potential of Cow urine as an extraction medium and subsequent impact on the efficacy of its extract.

Microbial biotransformation employs microorganisms for achieving the desired conversion of various substrates. Microorganisms can catalyze the reactions which otherwise are difficult to carry out. The success of microbial biotransformation depends greatly on proper selection of microorganisms. The well known enrichment techniques are used for isolation of potent microbial agents. These often employ enrichment cultures obtained from soils, decomposing materials, sewage plants, river water or factory grounds etc. The sample is incubated in a growth medium containing

substrate to be transformed as sole carbon source. Microorganisms employ both constitutive as well as inducible enzymes to degrade and synthesize a great variety of compounds. The discovery of the phenomenon of microbial biotransformation of complicated organic compounds provided impetus to employ microorganisms in the production of novel agents. Gradually, the methods of preparing new derivatives of known substrates were applied to modify organic compounds like Carbohydrates, steroids, sterols, terpenoids, flavonoids, alkaloids, antibiotics, amino acids etc. Such alteration encompasses many changes in the substrate moiety by the addition, degradation or modification. When basic structure of resultant molecule remains unaltered, the microbial biotransformation can be applied for formation of desired derivatives of precursor.

Traditionally, cow urine itself has some medicinal properties and employing it in the process of extraction through biotransformation is envisaged to have positive improvement in the original potency of the herb under study.

Material and methods:

Plant Material

Procurement of *Embellica officinalis* fruits

Fruits of *Embellica officinalis* were procured from the local market of Nagpur. Cow urine was procured from Govigyan Anusandhan Kendra, Dewlapar, Nagpur

Authentication

The fruits were authenticated by Dr. Dongarwar, Department of Botany, R.T.M. Nagpur University, Nagpur, with the herbarium voucher specimen number 9764 .

Preparation of Extract

The collected fruits about 200gm. were macerated in cow urine (1 Lit.) for time period of 28 days [13].After the completion of this time period, the biotransformed material (Extract) was harvested, lyophilized, powdered (20 Gm.) which was then used for further studies.

Preparation of Aqueous Extract for comparative study

The collected fruits about 200gm. were macerated in water (1 Lit.) for time period of 28 days. After the completion of this time period, the biotransformed material (Extract) was harvested, lyophilized, powdered (20 Gm.) which was then used for further studies.

Maintenance of Cell culture

The A549 cell lines, LN Cap- FGC cell lines and MDA-MB cell lines were maintained at The Maratha Mandal Dental College, Belgaum , Karnataka. Cell lines were grown in Minimal essential medium (MEM) supplemented with 4.5 g/L glucose, 2 m ML-glutamine and 5% fetal bovine serum (FBS) (growth medium) at 37 ^{0}C in 5% CO_2 incubator.

MTT Assay (Micro Titer Assay)

The *in vitro* MTT assay method was used to determine the inhibitory effects of test compounds on cell growth. The trypsinized cells from T-25 flask were seeded in each well of 96-well flat-bottomed tissue culture plate at a density of 5x10^{3}cells/well in growth medium and cultured at 37 ^{0}C in 5% CO_2 to adhere. After 48 hr incubation, the supernatant was discarded and the cells were pre-treated with growth medium

and were subsequently mixed with different concentrations of test samples (10, 20 and 30 µg) in triplicates to achieve a final volume of 100 µl and then cultured for 48 hr. The compound was prepared as 1.0 mg/ml concentration stock solutions in PBS. Culture medium and solvent were used as control and blank respectively. Each well then received 5 µl of fresh MTT (5mg/ml in PBS) followed by incubation for 2 hr at 37 ^{0}C. The supernatant growth medium was removed from the wells and replaced with 100 µl of DMSO to solubilise the colored Formosan product. After 30 min incubation, the absorbance (OD) of the culture plate was read at a wavelength of 492 nm on an ELISA reader [14].

The percentage inhibition of cancer cell lines was calculated with the following formula % inhibition = 100-(At-Ab) / (Ac-Ab) × 100

Where, At: Absorbance of test,

Ab: Absorbance of blank,

Ac: Absorbance of control.

Result and discussion:

The effect of biotransformed material (extract) of *Embellica officinalis* was studied using MTT assay at a dose of 10, 20, 30 µg and Vincristine was used as a standard at a dose of 90 µg indicate the efficacy of microbial biotransformed material against cancerous cells. Hence by

Cytotoxic properties of biotransformed material (extract) of *Embellica officinalis* against A549 cell lines-

The IC50 value of *Embellica officinalis* against A549 cell lines was found to be Less than 10 µg and the results are provided in Table 1.

Cytotoxic properties of biotransformed material (extract) of *Embellica officinalis* against LN Cap-FGC cell lines-

The IC50 value of *Embellica officinalis* against LN Cap-FGC cell lines was found to be 10 µg and the results are provided in Table 2.

Cytotoxic properties of biotransformation material (extract) of *Embellica officinalis* against MDA-MB cell lines-

The IC50 value of *Embellica officinalis* against MDA-MB cell lines was found to be 10 µg and the results are provided in Table 3.

Table. 1-Cytotoxicity effect against A549 cell lines

Sr. No.	Name of drug	Concentration (µg)	O.D. at 492nm	%of cell lysis	IC50
1.	Embellica officinalis Biotransformation material (extract)	10	0.719	75%	<10 µg
		20	0.782	>75%	
		30	1.226	100%	
2.	Control	-	0.240	No lysis	-
3.	Vincristine	90	1.117	90%	-

Table. 2-Cytotoxicity effect against LN Cap-FGC cell lines (LN Cap-FGC–Humancarcinoma Prostate)

Sr. No.	Name of drug	Concentration (µg)	O.D. at 492nm	%of cell lysis	IC_{50}
1.	*Embellica officinalis* Biotransformation material (extract)	10	0.578	50%	10 µg
		20	0.768	75%	
		30	1.084	100%	
2.	Control	-	0.321	No	-
3.	Vincristine	90	1.117	90%	-

Table. 3-Cytotoxicity effect against MDA-MB cell lines

Sr. No.	Name of drug	Concentration (µg)	O.D. at 492nm	%of cell lysis	IC_{50}
1.	*Embellica officinalis* Biotransformation material (extract)	10	0.622	50%	10 µg
		20	0.764	75%	
		30	0.940	100%	
2.	Control	-	0.334	No	-
3 .	Vincristine	90	1.117	90%	-

Comparative results for Aqueous Extracts-

Table.4-Cytotoxicity effect against A549 cell lines

Sr. No.	Name of drug	Concentration (µg)	O.D. at 492nm	%of cell lysis	IC_{50}
1.	*Embellica officinalis* Aqueous extract	10	0.719	No Lysis	---------
		20	0.782	No Lysis	
		30	1.226	No Lysis	
2.	Control	-	0.240	No lysis	-
3.	Vincristine	90	1.117	90%	-

Table.5-Cytotoxicity effect against LN Cap-FGC cell lines (LN Cap-FGC-Humancarcinoma Prostate)

Sr. No.	Name of drug	Concentration (µg)	O.D. at 492nm	%of cell lysis	IC_{50}
1.	*Embellica officinalis* Aqueous extract	10	0.578	No lysis	-------
		20	0.768	No lysis	
		30	1.084	No lysis	
2.	Control	-	0.321	No lysis	-
3.	Vincristine	90	1.117	90%	-

Table.6-Cytotoxicity effect against MDA-MB cell lines

Sr. No.	Name of drug	Concentration (µg)	O.D. at 492nm	%of cell lysis	IC_{50}
1.	*Embellica officinalis* Aqueous extract	10	0.622	No lysis	--------
		20	0.764	No lysis	
		30	0.940	No lysis	
2.	Control	-	0.334	No lysis	-
3 .	Vincristine	90	1.117	90%	-

Conclusion:

The anti-cancer studies of the extract of *Embellica officinalis* obtained after biotransformation using cow urine as an extraction medium on A549 cell lines, LN Cap-FGC cell lines and MDA-MB cell lines was carried out by using MTT cell growth inhibition assay. The results showed that the maximum percentage inhibition of cancer cell lines by *Embellica officinalis* biotransformed extract was found to be 50% at a dose of less than 10 µg for A549 cell lines, 50% at a dose of 10 µg for LNCap-FGC cell linesand50% at a dose of 10 µg for MDA-MB cell lines. Hence biotransformed material (extract) of *Embellica officinalis* obtained by using cow urine as medium for extraction can be used as a potent anti-cancer agent whereas the aqueous extract of same plant material doesn't show any activity.

Acknowledgement:

We wish to thank the Govigyan Anusandhan Kendra, Dewlapar, Nagpur, for providing fresh cow urine and Maratha Mandal Dental college, Belgaum , Karnataka, who gave support to carry the *in vitro* anti-cancer studies.

References:

1. **Akihisa T.(1996).** Triterpene alcohols from the flowers of compositae and their anti-inflammatory effects, Phytochemistry, 1996; 43(6), 1225-1260.

2. **Manoharan et al., (2012).** In Vivo and In vitro antitumoractivity of *Jasminum samba* (linn) AIT Oleaceae flower against Dalton's ascites lymphoma induced Swiss albino mice. International Journal of Pharmacy and Pharm aceutical Sciences 2012; 4(1): 144-147.

3. **Mangale S.M., Chonde S.G. and Raut P.D. (2012)**. Use of *Moringaoleifera* (drumstick) seed as natural absorbent and an antimicrobial agent for ground water treatment, Res J Recent Sci. 2012; 1(3): 31-40.

4. **Mondal D. andMondal T. A. (2012).** Review on efficacy of *Azadirachta indica* A. juss based biopesticides: an Indian perspective, Res J Recent Sci. 2012; 1(3): 94-99.

5. **Jocelyn P.C. (1962).** The effect of glutathione on protein sulphydryl groups in Rat-liver homogenates, Biochem J1962; 85: 480-485.

6. **Zitterl-Eglseer K., Sosa S., Jurenitsch J., Schubert-Zsilavecz M., Della Loggia R., Tubaro A., Bertoldi M. and Franz C.(1997).** Anti-edematous activities of the

main triterpendiol esters of marigold (*Embellica officinalis*L.) J Ethnopharmacol1997; 57:139–144.

7. **Preethi K.C., Kuttan G. and Kuttan R. (2006).** Antioxidant potential of *Embellica officinalis* flowers in vitroandin vivo. Pharmaceutical Biol 2006; 44(9):691–697.

8. **Della Logia R., Tubaro A., Sosa S., Becker H., Saar S. andIssac O. (1994).** The role of triterpenoids in the topical anti-inflammatory activity of*Embellica* fruits. Planta Med 1994; 60: 516–520.

9. **Dumenil G., Chemli R. and Balausard G .(1980).** Evaluation of antibacterial properties of *Embellica officinalis* flowers and mother homeopathic tinctures of*Embellica officinalis*. Ann Pharma Fran 1980; 38:493–499.

10. **Kasiram K., Sakharkar P. R. andPatil A.T. (2000).** Antifungal activity of*Embellicaofficinalis*.Indian J Pharm Sci. 2000; 6:464–466.

11. **Barbour E.K., Sagherian V., Talhouk S., Talhouk R., Farran M.T., Sleiman F.T. and Harakeh S., (2004).** Evaluation of homeopathy in broiler chickens exposed to live viral vaccines and administered *Embellica officinalis* extract. Med SciMonit2004; 10(8):281–285.

12. **Boucaud-Maitre Y., Algernon O. and Raynaud J. (1988).** Cytotoxic and antitumoral activity of *Embellica officinalis* extracts. Pharmazie1988; 43:220–221.

13. **Murkute A. V., Sahu M. S., Mali P. Y. and Rangari V. D. (2010).** Development and Evaluation of Formulations of Microbial Biotransformed Extract of Tobacco Leaves for Hair Growth Potential, Pharmacognosy Research 2010; 2 (5): 300-303.

14. **Meenavangalapati, Surya Prakash D. andSreeSatyanandam, (2013).** In - Vitro Anti - Cancer Studies of Chebulinic acid on colon adenocarcinoma Ht- 29 cell lines. Int J Pharm PharmSci, 2013; 5(2): 582-583.