WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 4, 1537-1544. Research Article ISSN 2277– 7105

PREFORMULATION STUDIES & PHYTOCHEMICAL ANALYSIS OF ETHANOLIC EXTRACT OF *EMBILICA OFFICINALIS* AND *GLYCINE MAX MERRILL.*

Suchita Wamankar, Anshita Gupta and Chanchal Deep Kaur*

Shri Rawatpura Sarkar Institute of Pharmacy, Kumhari, Durg, Chhattisgarh.

Article Received on
03 Feb 2015,

Revised on 28 Feb 2015,
Accepted on 25 March 2015

***Correspondence for Author**
Dr. Chanchal Deep Kaur
Shri Rawatpura Sarkar Institute of Pharmacy, Kumhari, Durg, Chhattisgarh.

ABSTRACT

Embilica officinalis (Euphorbiacea) and ***Glycine max Merrill*** (Papllionaceae) are the most common medicinal plants and it's having strong anticancer properties against ovarian cancer. It having a great curing properties for treatment of ovarian cancer. The dried fruits and seeds were identified and collected from, Raipur, Chhattisgarh. The aim of present work is focused on analysis phytochemical properties and antioxidant properties of Embilica officinalis and Glycine max Merrill. In the phytochemical analysis estimation of phytoconstituents glycosides, alkaloids, tannin, saponin and flavonoids were estimated for crude powder. The antioxidant properties of ethanolic extract were analysis by DPPH percentage free radical inhibition activity. By this analysis were found Embilica officinalis fruit extract have greater antioxidant activity comparsion then Glycine max Merrill ethanolic extract. In future work design novel drug delivery system of combination with ethanolic extract of Embilica Officinalis and Glysine max Merrill for effective activity against ovarian cancer.

KEYWORDS: phytochemical, antioxidant, ethanolic, DPPH.

INTRODUCTION

Ovarian Cancer is the fourth most common type of cancer in India. Among 26.1% female are suffering with ovarian cancer in India.[1] Ovarian cancer is a type of cancer when cancer begins from ovaries. It is known as one of the leading cause of death in women.[2] Women having two ovaries that located in pelvis and both are on each side of uterus. The ovaries main function is to produce female hormones and eggs.[3] Increase in oxidative stress causes high risk of ovarian cancer. In patients where increased levels of plasma lipid per-oxidation

and low levels of SOD, Vitamin C ,CAT and Vitamin E are observed as compared to normal subject, have higher chances of ovarian cancer.[4] To maintain the harmonization between oxidants and anti-oxidants, natural antioxidants such as vincristine, vinblastine, ellagic acid , qurecetin, genistein, andographides and podophyllotoxin are used which also play crucial role as anticancer agents.[5] We selected some antioxidants which have antioxidant properties and can be used for fightining against ovarian cancer. Dried Amla obtained from *Embilica officinalis* of the family Euphorbiaceae and Ground Soyabean obtained from *Glycine max Merrill* of the family Papllionaceae, are most common herbs widely consumed beverages in worldwide. The *Embilica officinalis* fruit contains gallic acid, qurecetin, pectin, tannins, vitamin C and phyllaemblic compounds and also contains various polyphenolic compounds, ellagic acid, gallic acid, chebulinic acid, quercetin, and chebulagic acid.[6] The *Glycine max Merrill* is a soya isoflavones contain genistein and diadzein. Ground soya bean contain isoflavones and other glycosides. Both herbs are also rich in flavonoidal compounds. All flavonoids have an inhert tendency to act as free radical scavenger and hence restore the cellular capacity of reformation naturally.This is the reason why most of the flavanoidal drugs are being for treatment of different types of cancer. Dried Amla is also used to treat fever, as diuretic, laxative, liver tonic, refrigerant, stomachic, restorative, anti-pyretic, hair tonic, to prevent ulcer and dyspepsia.[7] Similarly, Ground Soya bean is also used as nutrient and supplement.[8]

The aim of our study was to produce ethanolic extracts of dried amla fruit and ground soyabean and to carry out their preformulation studies & phytochemical analysis. Our approache is to develop novel drug delivery system of herbal extract and comparison with pure phytoconstitutent against ovarian cancer.

MATERIALS AND METHODS

Dried *Embilica officinalis* fruit and *Glycine max Merrill* was purchased from authentic supplier of herbal products of Raipur District. 2-2-Diphenyl-1-Picrylhydrazyl (DPPH) was purchased from allied scientific laboratories, Nagpur and petroleum ether 60-80ºC, Ethanol was purchased from supplier. L-ascorbic acid was from institution lab. Depending upon exhaustive literature review dried *Embilica officinalis* fruit and *Glycine max Merrill* were selected for preparation of extracts.[9]

Preparation of Extract

The dried material of *Embilica officinalis* and *Glycine max Merrill* was cleaned and then crushed for powder. Exactly 60gm of each herb was weighed and defatted with 200 ml of petroleum ether 60-80ºC and then air dried. After that herbs were extracted with ethanolic solvent for 24 hr by a continuous hot extraction method, until complete exhaustion of the drug occurs using a soxhlet apparatus. A deep brown viscous residue obtained having characteristic odour. Further the solvents were evaporated to dryness.

Estimation of physicochemical parameters

The determination of various physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, water soluble extractive value, alcohol soluble extractive value, swelling index, foaming index, moisture content, ash value, pH were calculated as per Indian Pharmacopoeia.[10]

Extraction value: Extraction value was determined in different solvents as Ethanol, Chloroform and Petroleum ether 60-80 °C. Weighed the empty weight of every conical flask where sample was kept. Again weighed the exactly 2 gm of herbs powder and take in conical flask were 10 ml of solvents in different conical flask. Tightly close the mouth of conical flask by cotton and aluminium foil paper and stand for 24 hr and observed the first solvent was evaporate and calculated the extraction value.

Table 1. Extraction value of *Embilica officinalis* herb

Solvent	Extraction value
Chloroform	0.07
Petroleum ether	0.04
Ethanol	0

Table 2. Extraction value of *Glycine max Merrill* herb

Solvent	Extraction value
Chloroform	0.03
Petroleum ether	0.06
Ethanol	0

Preliminary phytochemical screening

For preliminary phytochemical screening, 100 g of powder drug was extracted with ethanol. The mother extract obtained from successive solvent extraction were then subjected to various qualitative chemical tests to determine the presence of various phytoconstituents like

alkaloids, glycosides, carbohydrates, phenolics and tannins, phytosterols, fixed oils and fats, proteins an a amino acids, flavonoids, saponins, gums and mucilages etc.[11-13]

Percentage of Free radical inhibition by DPPH

0.004% DPPH solution was prepared using 20 mg DPPH with 500ml of ethanol. Then prepared stock solution by using 10 mg of ethanolic extract with 10 ml of ethanol. Than prepared different dilution 0.25µg/ml, 0.50µg/ml, 0.75µ g/ml, 1.00µg/ml, 1.25µg/ml of working solution and makeup it into 10 ml with help of 0.004% of DPPH solution in volumetric flask. Tightly Close the flask with cap. Keep it 30 min in a incubator for 20ºC .after 30 min take absorbance at 517 nm in a UV Shimadzu spectrophotometer. Calculate % free radical inhibition and compare with Ascorbic acid as standard.[15][16]

$$\% \text{ free radical inhibition} = \frac{A_{CONTROL} - A_{TEST}}{A_{CONTROL}} \times 100$$

Where $A_{CONTROL}$ is the absorbance of the control reaction and A_{TEST} is the absorbance in the presence of the sample of the extracts. Then Table 6 shows that the volume of ascorbic acid and extract taken for the present study. And plotted the graph for comparision of extract from L-Ascorbic acid[16-17] (figure 1).

Preliminary phytochemical screening

For preliminary phytochemical screening, 100 g of powder drug was extracted with ethanol. The mother extract obtained from successive solvent extraction were then subjected to various qualitative chemical tests to determine the presence of various phytoconstituents like alkaloids, glycosides, carbohydrates, phenolics and tannins, phytosterols, fixed oils and fats, proteins an a amino acids, flavonoids, saponins, gums and mucilages etc.[13]

RESULT AND DISCUSSION

Physio-chemical Parameters

The determination of physico-chemical parameter is important in determination of adulterants and improper handling of drugs. Table- 4 shows the result of various physico chemical parameter of powdered drug carried out using standard methods. Moisture content of drugs could be at minimal level to discourage the growth of bacteria, yeast or fungi during storage. Ash values used to determine quality and purity of crude drug. It indicates the presence of various impurities like carbonate, oxalate and silicate.[9] The acid insoluble ash consist mainly silica and indicate contamination with earthy material. The water soluble ash is used to

estimate the amount of inorganic elements present in drugs. The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent.[10]

Table No.3 Organoleptic Evaluation[11]

S.No.	Oraganoleptic Parameters	*Embilica officinalis*	*Glycine max Merrill*
1.	Colour	Blackish	Yellowish
2.	Odour	Characteristic	Characteristic
3.	Taste	Sour	Pungent

Table no .4 Physical Evaluation Parameters[11],[12],[13]

Extractive Values	Values (%)(w/w)	Values (%)(w/w)
Loss on Drying	43.50%	28.50%
Swelling Index	7.5	2.5
Total Ash Value	3.2%	5.3%
Acid Soluble Ash Value	0.8%	1.3%
Water Insoluble Ash Value	4.9%	3.7%

Phytochemical Analysis of Dried Powder of *Embilica officinalis* & *Glycine max Merrill*

The powder drug with different chemical reagents show different color when seen on naked eye. The different colour oberved shows presence of different type of phytoconstituent. Many drugs fluorescence when their powder is exposed to ultraviolet radiation. It is important to observe all materials on reaction with different chemical reagents under U.V. light. The fluorescence characteristics of powdered drug were studied under U.V. light after treating with different chemical reagents are reported.[13] (Table No.4).

Table 5. Phytochemical screening of herbs extracts

S.No	Test	Amla extract	Soyabean extract
a)	**Test for alkaloid (Hager's test)**	No precipitate form it not show present of alkaloid	No precipitate form it not show present of alkaloid
b)	**Test for Saponin**	No foaming produce it not show present of Saponin	No foaming produce it not show present of Saponin
c)	**Test for Glycosides** a)Killer Killani Test b) Bromine Water Test	 It not show present of glycosides No yellow precipitate form it not show present of glycosides	 It not show present of glycosides No yellow precipitate form it not show present of glycosides
d)	**Test for flavonoids** a) ferric chloride test	Blackish red colour form it show present of flavonoids	Blackish red colour form it show present of

	b) Alkaline Reagent test c) Lead Acetate Solution test	Colourless it show present of flavonoids Yellow precipitate form it show present of flavonoids	flavonoids Colourless it show present of flavonoids Yellow precipitate form it shows the presence of flavonoids

The results are expressed as mean ± S.E.M. The statistical analysis was performed by analysis of variance (A.N.O.V.A) by Dunnett's Multiple Comparison test. Mean ± SEM, * $p<0.05$, ** $p<0.001$.

Free radical Inhibition of Dried Powder Extract of *Embilica officinalis* & *Glycine max Merrill*

Table no.6 Percentage of free radical inhibition of herbs extract

Concentration of ascorbic acid	% free radical inhibition of ascorbic acid	% free radical inhibition *Embilica officinalis* fruit extract	% free radical inhibition *Glycine max Merrill* extract
0.25µg/ml	98.27	95.57	19.22
0.50µg/ml	95.28	94.91	29.80
0.75µg/ml	98.09	91.72	35.99
1.00µg/ml	98.26	94.36	32.70
1.25µg/ml	98.33	93.81	46.06

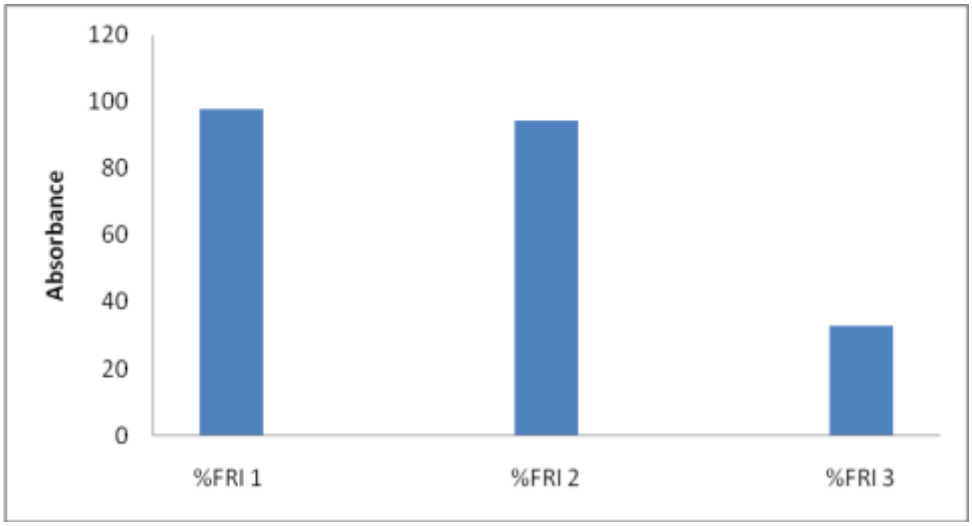

Figure.1 Potency of % free radical inhibition activity on free radical DPPH of L-Ascorbic acid. Bars represent the (standard error of experiments carried out in *Embilica officinalis* and *Glycine max Merrill*. 1 represent for L-Ascorbic acid, 2 represent for *Embilica officinalis* extract and 3 represent for *Glycine max Merrill* extract.

The results are expressed as mean ± S.E.M. The statistical analysis values Statistical tests as well as mean and S.E.M calculations and graphical representation of result were performed.

CONCLUSION

The above study laid us to drive a conclusion that ethanolic extract of *Embilica officinalis* was more effective antioxidant potential as compare with the ethanolic extract of *Glycine max Merrill*. The activity spectrum exhibited by *Embilica oficinalis* extract was twice that of *Glycine max Merrill*. This may be due to the fact that *Embilica officinalis* is a natural source of Vitamin – C i.e ascorbic acid. The level of free radical inhibition exhibited by *Glycine max Merrill* was although lesser but can be used in combination with *Embilica officinalis* for more profound effect. During the course of our study we found that both the herbs were compatible with each other. Hence, it can be postulated that *Embilica officinalis* and its combination with *Glycine max Merrill* can be further designed using an appropriate delivery system for treating ovarian cancer.

Conflict of Interest: None

REFERENCES

1. Gautam.S, Bhatt.MLB, Singh . R, Mehrotra .S, Singh. U, Saxena .J.K and Singh*****.R.K; **"**Impact of therapeutic intervention on oxidants and antioxidants status in patients with ovarian malignancy"; Biomedical Research, 2011; 22(3): 259-262.
2. Nandagudi Srinivasa Murthy, S. Shalini, G. Suman, Srikantaiah Pruthvish,Aleyamma Mathew; Changing Trends in Incidence of Ovarian Cancer - the Indian Scenario; Asian Pacific Journal of Cancer Prevention, year- 2009; 10: 1025-1030.
3. Cancer; American cancer society; year-2014.
4. Himmat Singh Chawda ; Prospective Study of Antioxidants, Its Mechanism and Potential Role in Cancer; International Journal of Research in Pharmaceutical and Biomedical Sciences, year -2011; 2(3): 888-894.
5. Pandey. G; Some important anticancer herbs: a review; International Research journal of pharmacy, year- 2011; 2(7): 45- 52.
6. Singh .E, Sharma. S, Pareek .A, Dwivedi .J, Yadav .S and Sharma .S; Phytochemistry, traditional uses and cancer chemopreventive activity of Amla (*Phyllanthus emblica): The Sustainers';* Journal of Applied Pharmaceutical Science, Year-2011; 02(01): 176-183.
7. Udgata .J and Naik. S.N; Soyabean isoflavones: Remedial nutraceuticals in Indian prespective ; journal of scientific and industrial research, January 2007; 66: 11-18.
8. Alka Sawarkar*, C.R. Jangde, P.D.Thakre, Ranu Kadoo and Sushma Shelu, Analgesic Activity of Hibiscus rosa sinensis Linn in Rat, Veterinary World, 2(9): 353-354.

9. Sukhdev Swami Handa, Suman Preet Singh Khanuja, Gennaro Longo, Dev Dutt Rakesh. Extraction technologies for medicinal and aromatic plants**,** International centre for science and high technology, 2008.
10. Gupta V1*, Bansal P1, Garg A2, Meena AK1;Pharmacopoeial Standardization of Hibiscus rosa sinensis Linn., *International Journal of Pharmaceutical and Clinical Research*, 2009; 1(3): 124-126.
11. Heinz Lüllmann, M.D. ,Klaus Mohr, M.D. ,Lutz Hein, M.D.; Color Atlas of Pharmacology; 3rd edition, revised and expanded; Thieme Stuttgart· New York.
12. Peter J Houghton; Evaluation of Herbal Medicinal Products; Perspectives on quality, safety and efficacy; First published 2009; Published by the Pharmaceutical Press, London, 3-14, 327.
13. V. M. Jadhav *, R. M. Thorat1 ,V.J. Kadam 1& N. S. Sathe2, Hibiscus rosa sinensis Linn – ‘‘Rudrapuspa’’ : A Review; Journal of Pharmacy Research, 2009; 2(7): 1168-1173
14. Ak Meena*, Arjun Singh, Mm Rao ; Evaluation Of Physicochemical And Preliminary Phytochemical Studies On The Fruit Of Emblica Officinalis Gaertn ; Asian Journal of Pharmaceutical and Clinical Research; 20103; 3.
15. K.H. Khan; Roles of *Emblica officinalis* in Medicine - A Review; Botany Research International, 2009; 2(4): 218-228.
16. Mhaskar, K.S. and Bletter, E., Eds., In; Kirtikar and Basu´s Illustrated Indian Medicinal plants, Sri Satguru Publications, New Delhi, 1989; 462.
17. Deep Chanchal and Saraf Swarnlata; Herbal Photoprotective Formulations and their Evaluation ; The Open Natural Products Journal; year- 2009; 2: 71-76.
18. Sarabjot Kaur1* and Poonam Mondal, Study of Total Phenolic and Flavonoid Content, Antioxidant Activity and Antimicrobial Properties of Medicinal Plants, Journal of Microbiology & Experimentation, Volume 1 Issue 1 – 2014; 1-6.