International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 26-11-2020; Accepted: 13-12-2020: Published: 24-12-2020
www.botanyjournals.com
Volume 5; Issue 6; 2020; Page No. 704-709

Comparative *in-vitro* antioxidant activity of fruit extracts of *Embelica officinalis* Gaertn and drug likeness profile of selected phytoconstituents

Shailendra Sanjay Suryawanshi[1*], Snehal Maruche[1], Pruthviraj Patil[1], MS Palled[1], Yogesh Pancham[2]
[1] Department of Pharmaceutical Chemistry, KLE College of Pharmacy, Belagavi, KLE Academy of Higher Education and Research, Belagavi, Karnataka, India
[2] Department of Pharmaceutical Quality Assurance, KLE College of Pharmacy, Belagavi, KLE Academy of Higher Education and Research, Belagavi, Karnataka, India

Abstract
In the present research work an attempt has been made to compile the drug likeness profile of selected phytoconstituents present in fruits of *Embelica officinalis* Gaertn and to comparatively investigate *in-vitro* antioxidant activity of various fruit extracts. Online web server MolSoft and mol inspiration were used for prediction of drug-likeness properties of selected phytocompounds. Extraction of phytochemicals from fruits of *Embelica officinalis* Gaertn was carried out by maceration process using water, ethanol and methanol as solvents. All the extracts were investigated for the presence several phytochemicals using qualitative chemical test. Antioxidant activity of aqueous, ethanolic and methanolic extract was performed by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method using Quercetin as standard. Results of present investigation showed the drug like score of selected Phytoconstituents. Aqueous, ethanolic and methanolic extracts of fruit yields variety of phytochemicals and have potent antioxidant activity. Ethanolic extract found to show better activity that aqueous and methanolic extract.

Keywords: antioxidant activity, quercetin, drug likeness, Molsoft, phytocompounds

Introduction
Plants are the great source of sophisticated natural products and play important role in the new drug development [1-4] which were evaluated via in silico molecular docking, *in vitro* enzyme inhibitory assay and enrichment analysis. In worldwide approximately 80% of world peoples are using traditional medicine for their primary health care [5-6]. *Emblica officinalis* Gaertn (Amla) family Euphorbiaceae is a prestigious herb used to prepare Ayurvedic medicines because of its miraculous actions and used as Rasayan as mentioned in Charak Samhita [7]. Amla is supposed to rejuvenate all the organ systems of the body, provide strength and wellness. It keeps us away from all the diseases by boosting our immune system [8, 9].
Chemically the fruits of Amla found to possess wide range of phytochemicals. They are rich in tannins, emblicanin A and B, which have antioxidant properties. Emblicanin A on hydrolysis gives gallic acid, ellagic acid and glucose whereas Emblicanin B on hydrolysis gives ellagic acid and glucose. They are also found to possess carbohydrates, fats, proteins, amino acids, minerals, ascorbic acid, β-carotene, chebulagic acid, chebulaginic acid, chebulic acid, chebulinic acid, corilagic acid, corilagin, ellagic acid, emblicol, flavonoids, gallic acid, myristic acid, niacin, phyllantidine, phyllantine, phyllemblic acid, phyllemblin, phyllemblinic acid, quercetin, riboflavin, rutin and terchebin. [10, 11, 12].
Fruits have many traditional and biological effects. Fresh fruits are found to show refrigerant, diuretic, and laxative, carminative and stomachic activities. Dried fruit is sour and astringent. Amla fruit paste is main ingredient of Chyawanprash, a popular Ayurvedic tonic. Amla is known as Amritphale in Sanskrit, which literally means the fruit of heaven or nectar fruit and is the richest source of Vitamin C and hence it have great antioxidant activities [13-21]. The picture of fruits is presented in Figure 1.

Fig 1: Fruits of *Embelica Officinalis* Gaertn

Solvents play an important role in the extraction process, as different solvents have ability to extract different constituents from crude drug. Various studies have been reported on antioxidant activities of fruits of *Embelica officinalis* Gaertn. But no comparative antioxidant activity evaluation studies have been reported. Hence in the present research an attempt has been made to investigate anti-oxidant activity of fruit extracts of *Embelica officinalis* Gaertn using different solvents.
The Lipinskies rule of five found to be as useful in defining the drug like properties of molecules. The rule of five criteria was originally proposed by Lipinski and coworkers. In the drug discovery field, the rule of five predicts that poor absorption is more likely when there are more than ten H-

bond acceptors, five H-bond donors, molecular weight is more than 500, and the calculated Log P is more than five [22].

Lipinski's rule of five is also known as Pfizer's rule of five or the rule of five (RO5). R05 is a rule of thumb to evaluate drug likeness or determine a molecule with a certain biological activities. The rule is important to keep in mind during drug discovery when a pharmacologically active lead structure is optimized step-wise to increase the activity and selectivity of the compound as well as to ensure drug-like physicochemical properties are maintained as described by Lipinski's rule. Candidate drugs that conform to the RO5 tend to have lower attrition rates during clinical trials and hence have an increased chance of reaching the market [23, 24]. Knowledge about the drug like properties of molecules plays an important role in the drug discovery. In the present study we have selected few important phytocompounds from fruits and its drug like properties have been determined and reported along with its antioxidant profile.

Materials and methods

Instruments and apparatus

Shimadzu UV Spectrophotometer (UV-1800) was used to record the absorbance samples prepared for *in-vitro* antioxidant activity. Hot air oven was used to dry the crude drug and extracts. Calibrated weighing balance was used in the study for weighing of crude drugs and extracts.

Solvents and reagents

All the solvents and reagents used for the study were pure and obtained from store house of KLE College of Pharmacy, Belagavi. Distilled water was collected from distillation unit from department of Pharmacognosy. Ethanol and methanol were of Merck Ltd. All the test reagents used for phytochemical analysis were prepared freshly and used from department of Pharmacognosy.

Software

Molsoft and mol inspiration were used to determine the drug likeness profile of phytochemicals. For recording of absorbance of samples UV probe software in UV-Spectrophotometer was used.

Determination of Drug Like Properties of Selected Phytoconstituents

Online web server MolSoft was used for prediction of drug-likeness properties of Quercetin, Kaempferol, Emblicanin A, Emblicanin B, Phyllantidine, Chebulagic acid, Chebulinic acid, Puniglucanin, Vitamins C, Phyllanemblinin A, Ellagic acid, Hexahydroxydiphenic acid, Methyl gallate, Luteolin-7o-neohesperidoside and Corilagin**.** Drug-like properties were calculated based on the Lipinski rule of five [25]. The Canonical simplified molecular line-entry systems (SMILES) were retrieved from PubChem and submitted as input to mol inspiration and MolSoft web server to predict drug-like properties of compounds [26].

Collection of plant materials, authentication

The dried fruits of *Embelica officinalis* Gaertn were obtained from KLE Ayurveda Pharmacy, Belagavi. Dried fruits were grounded to get course powder. The powdered material was subjected to cold extraction (maceration) with water, ethanol and methanol.

Phytochemical extraction

Extraction of powdered material was carried out by using maceration process. Distilled water, ethanol and methanol were used as solvents for extraction. 10 gm of powdered material was extracted with 100 ml of distilled water, ethanol and methanol seperately. The content was shaken continuously during maceration period. After the period of maceration contents were filtered through muslin cloth and filtrate were evaporated to get thick and concentrated residue.

Phytochemical investigation

The qualitative tests were performed for detecting the presence of different phytoconstituents groups in the extract. Chemical test for alkaloids, glycosides, flavonoids, saponins, phenolic, tannins, terpeniods were performed as per standard procedures.

Evaluation of *in-vitro* antioxidant activity

The antioxidant activity of fruit extracts of *Embelica officinalis* Gaertn was evaluated against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical by measuring the absorbance at 518 nm using UV-Visible spectrophotometer. For the reference we have followed the method as mentioned in previously published work [27].

Preparation of working stock solution of extract

100 mg of aqueous, ethanolic and methanolic extract were weighed accurately and transferred into 100 mL volumetric flask seperately. Small amount of DMSO was added into each and volume was made up to the mark using analytical grade methanol. From the resulting stock solution different concentration ranging from 100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL and 500 µg/mL of respective extracts were prepared.

Preparation of standard stock solution

10 mg of standard Quercetin was weighed and accurately transferred into 100 mL volumetric flask. Volume was made up to the mark using methanol. From this stock further dilution were made to prepare working standards.

Preparation of dilutions for antioxidant activity

1 mL from each extract and 3 mL of methanol were mixed by 0.5 mL of 1.0 mM DPPH in methanol. 1 mL of working standard solution of Quercetin was mixed with 3mL of methanol and 0.5 mL of 1.0mM DPPH solution. 3 mL of methanol and 0.5 mL of DPPH was mixed and used as blank solution. All the prepared dilutions were allowed to react at room temperature for 30 minutes. All the samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the plot of inhibition percentage against extract concentration.

Results and Discussion

Drug Likeness Properties of Selected Phytoconstituents

The drug like properties and score of Quercetin, Kaempferol, Emblicanin A, Emblicanin B, Phyllantidine, Chebulagic acid, Chebulinic acid, Puniglucanin, Vitamins C, Phyllanemblinin A, Ellagic acid, Hexahydroxydiphenic acid, Methyl gallate, Luteolin-7o-neohesperidoside and Corilagin were presented in Table 1. Log P value play an important role in the absorption of drug. The LogP values of

selected constituents were presented in Figure 2. Graphical Presentation of drug likeness Score (DLS) of selected phytochemicals were presented in Figure 3.

Table 1: Drug Likeness Properties and Score of Selected Phytoconstituents

Sr. No	Compound Name	Molecular Weight	HBA	HBD	Log p	Drug Likeness Score
1	Quercetin	302.23 g/mol	7	5	2.11	0.93
2	Kaempferol	286.24g/mol	6	4	1.61	0.5
3	Emblicanin A	782.5 g/mol	22	12	-1.27	-0.05
4	Emblicanin B	780.5 g/mol	22	12	2.41	-0.87
5	Phyllintidine	233.26g/mol	4	0	1.85	-1.03
6	Chebulagic acid	954.7g/mol	13	27	0.22	0.58
7	Chebulinic acid	956.7 g/mol	27	13	-1.69	0.84
8	Puniglucanin	802.6 g/mol	23	14	-1.29	0.4
9	Vitamins C	176.12 g/mol	6	4	-2.4	0.84
10	Phyllanemblinin A	970.7 g/mol	28	14	-3.64	0.55
11	Ellagic acid	302.19 g/mol	8	4	1.02	-0.98
12	Hexahydroxydiphenic acid	338.22 g/mol	10	8	0.56	-0.34
13	Methyl gallate	184.15 g/mol	5	3	0.9	-0.49
14	Luteolin-7o-neohesperidoside	594.5 g/mol	12	9	-1.35	1.04
15	Corilagin	634.5 g/mol	18	11	-2.2	0.83

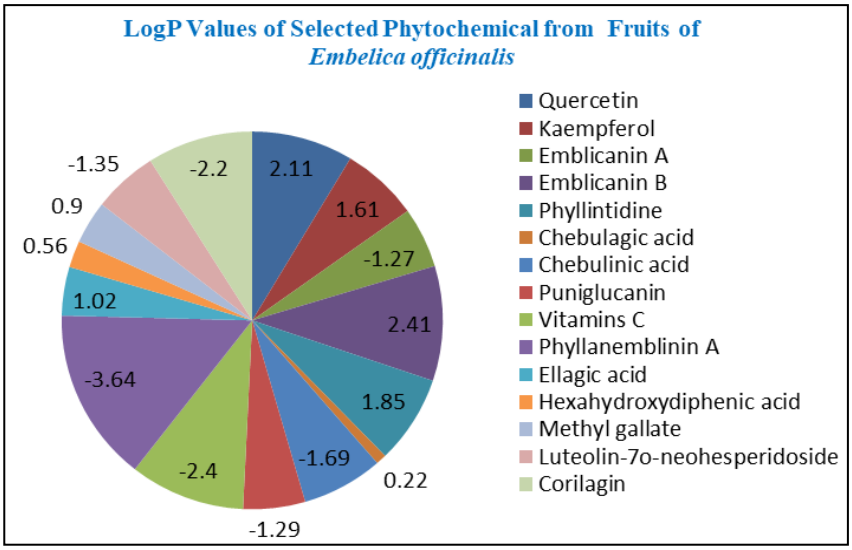


Fig 2: LogP Value of Selected Phytochemicals

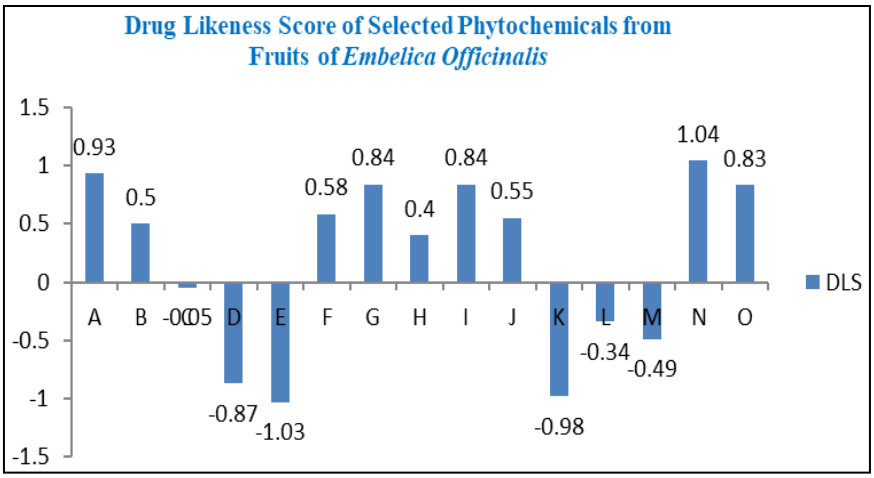


Fig 3: Drug Likeness Score of Selected Phytochemicals

Extraction of powdered drug

The dried fruits of *Embelica officinalis* Gaertn were obtained from KLE Ayurveda Pharmacy, Belagavi also authenticated with CRF code CRF/Auth/2019/200. The crude drug were powdered and subjected for extraction. The extraction of powdered material was carried out by maceration process. The extracts were concentrated dried and stored in well closed container for further studies. The colour, amount of extract obtained and percentage yield of extracts were presented in Table 2.

Table 2: Physical characteristics and % yield of Fruit Extracts of *Embelica officinalis*

Type of Extract	Colour of extract	State	Amount of Extract	% Yield
Aqueous Extract	Brownish green	Solid	1.5 gm	15%
Ethanolic Extract	Reddish brown	Semi-solid	1.2 gm	12%
Methanolic extract	Reddish brown	Semi-solid	0.8 gm	08%

Phytochemical analysis

Qualitative chemical tests were performed individually for the detection of each extract. The phytochemical composition of plant extract using different solvents showed their presence ion respective extract. The results were presented in Table 3.

Table 3: Phytochemical Screening of Fruit Extracts of *Embelica officinalis* Gaertn

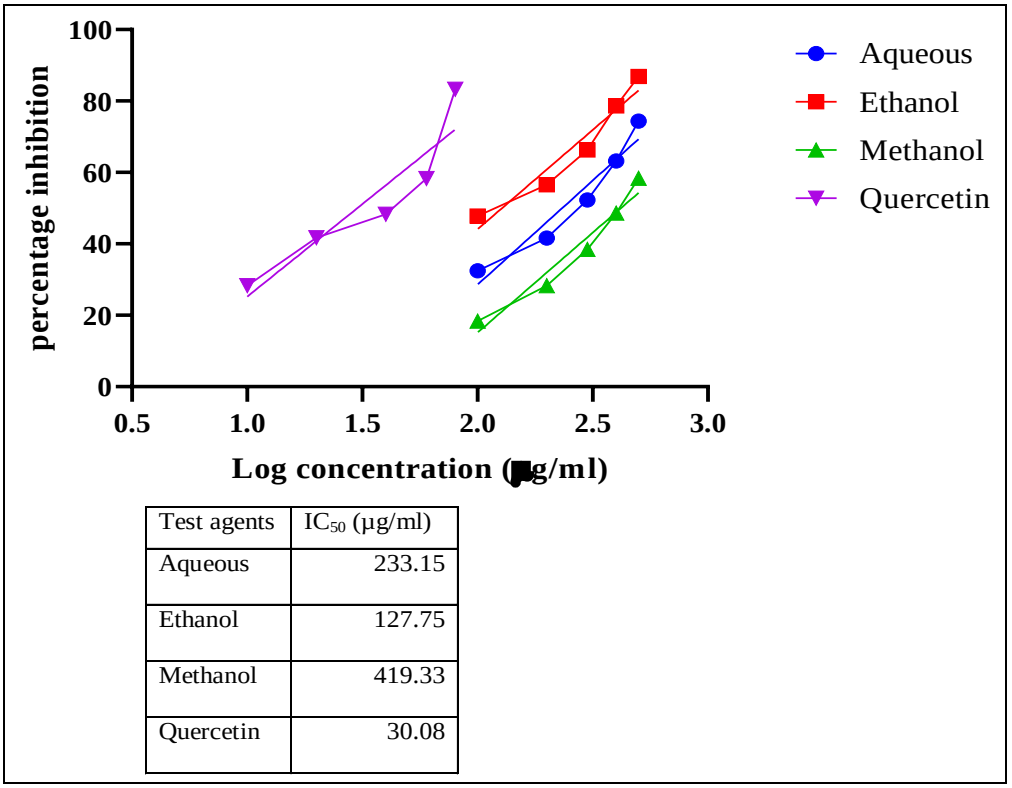
Sr. No.	Phytoconstituents	Aqueous Extract	Ethanolic Extract	Methanolic Extract
1	Carbohydrates	+ ve	-Ve	-Ve
2	Proteins	+Ve	+Ve	+Ve
3	Glycosides	-Ve	-Ve	-Ve
4	Steroids	-Ve	-Ve	-Ve
5	Tannins	-ve	+ve	-ve
6	Alkaloids	-ve	+ve	+ve
7	Triterpenoids	-Ve	-Ve	-Ve
8	Saponins	-Ve	-Ve	-Ve
9	Flavonoids	+ ve	+ve	-ve

Evaluation of antioxidant activity of extract

The anti-oxidant profiles of aqueous, ethanolic and methanolic extracts were reported in Table 4 and also outcome of the results were showed in Figure 4, 5 and 6. The mean % inhibition of aqueous, ethanolic and methanolic extract was found to be 52.81%, 67.22%, 38.41% respectively. IC_{50} values calculated for aqueous, ethanolic and methanolic extract were 233.15 µg/mL, 127.75 µg/mL, 419.33 µg/mL respectively. The value of standard Quercetin was found to be 30.08 µg/ IC_{50} mL. The results of antioxidant activity showed that ethanolic extract showed better antioxidant activity with IC_{50} Value 127.75 µg/mL with compared to aqueous and methanolic extract.

Table 4: Antioxidant Profile of Fruit Extracts of *Embelica officinalis* Gaertn

Sr. No.	Type of Extracts	Concentration	Mean % Inhibition	IC_{50} Values
1	Aqueous extract	100 - 500 µg/mL	52.81%	233.15 µg/mL
2	Ethanolic extract	100 - 500 µg/mL	67.22%	127.75 µg/mL
3	Methanolic extract	100 - 500 µg/mL	38.41%	419.33 µg/mL
4	Quercetin	10-50 µg/mL	82.43%	30.08 µg/mL



Test agents	IC_{50} (µg/ml)
Aqueous	233.15
Ethanol	127.75
Methanol	419.33
Quercetin	30.08

Fig 4: IC_{50} Values of Aqueous, Ethanolic and Methanolic Fruit Extracts

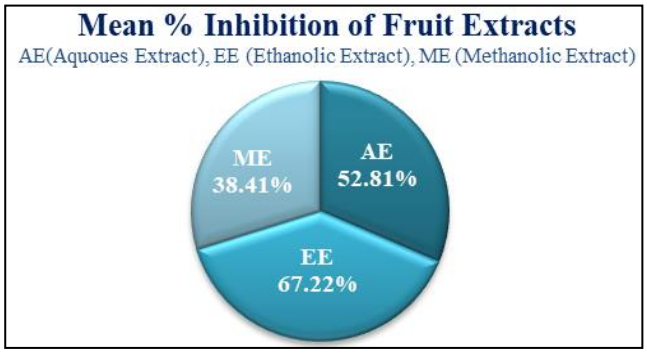


Fig 5: Mean % Inhibition of Fruit Extracts of *Embelica Officinalis* Gaertn

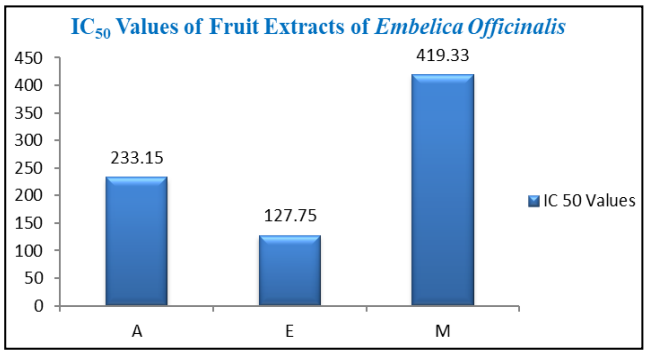


Fig 6: IC_{50} Values of Fruit Extracts of *Embelica Officinalis* Gaertn

Conclusion

The present investigation concluded that ethanolic fruit extract of *Embelica officinalis* Gaertn was found to show good antioxidant activity as compared to aqueous and methanolic extracts. In future there is a need to carry out the fractionation of ethanolic extract and evaluation of its antioxidant activity.

Acknowledgment

Authors are thankful to Principal Dr. Sunil S Jalalpure and Vice-Principal Dr. M. B. Patil, KLE College of Pharmacy, Belagavi for providing all the facilities to carry out the present research work and there constant support and guidance.

Reference

1. Khanal P, Patil B M. Gene set enrichment analysis of alpha-glucosidase inhibitors from Ficus benghalensis. Asian Pac J Trop Biomed. 2019; 9(6):263-70s
2. Khanal P, Patil BM. α-Glucosidase inhibitors from *Duranta repens* modulate p53 signaling pathway in diabetes mellitus. Adv Tradit Med. 2020. https://doi.org/10.1007/s13596-020-00426-w
3. Khanal P, Patil BM. Gene ontology enrichment analysis of α-amylase inhibitors from *Duranta repens* in diabetes mellitus. J Diabetes Metab Disord, 2020. https://doi.org/10.1007/s40200-020-00554-9
4. Ternikar SG, Patil MB, Pasha I, Khanal P. Gene set enrichment analysis of α-amylase and α-glucosidase inhibitors of *Cassia glauca*. J Diabetes Metab Disord, 2020. https://doi.org/10.1007/s40200-020-00538-9
5. Newman DJ, Cragg GM, Sander KM. The influence of natural products upon drug discovery. Natural product Res. 2000; 17:215-234.
6. Cragg GM, Byod MR, Khanna R, Mays TD, Mazan KD, Newman DJ *et al*. International Collaboration in drug discovery and development, the NCT experience. Pure Appl Chem. 1999; 71:1619-1633.
7. Ghoshal S, Triphati VK, Chauhan S. Active constituents of *Emblica officinalis*: Part 1- The chemistry and antioxidative effects of two new hydrolysable tannins, Emblicanin A and B. Indian J of Chem. 1996; 35B:941-948.
8. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. Nirali Prakashan. 2005; 268. ISBN No.81-85790-00-1.
9. Satyavati GV, Raina MK, Sharama M. Medicinal plants of India. Indian Council of Medical Research, New Delhi. 1976; 1:377.
10. Habib-ur-Rehman, Yasin KA, Choudhary MA, Khaliq N, Atta-ur-Rahman, Choudhary MI *et al*. Studies on the chemical constituents of *Phyllanthus emblica*. Nat Prod Res. 2007; 21(9):775-81.
11. El-Desouky SK, Ryu SY, Kim YK. A new cytotoxic acylated apigenin glucoside from *Phyllanthus emblica* L. Nat Prod Res. 2008; 22(1):91-5.
12. Alan. Siddha Medicinal Herbs as Cosmetics Ingredients. SPC, March 1994.
13. Scartezzini P, Antognoni F, Raggi MA, Poli F, Sabbioni C. Vitamin C content and antioxidant activity of the fruit and of the Ayurvedic preparation of *Emblica officinalis* Gaertn. J Ethnopharmacol. 2006; 104(1-2):113-8.
14. Rao TP, Sakaguchi N, Juneja LR, Wada E, Yokozawa T. Amla (*Emblica officinalis* Gaertn.) extracts reduce oxidative stress in streptozotocin induced diabetic rats. J Med Food. 2005; 8(3):362-8.
15. Sairam K, Rao CV, Babu MD, Kumar KV, Agrawal VK, Goel RK. Antiulcerogenic effect of methanolic extract of *Emblica officinalis*: An experimental study. J Ethnopharmacol. 2005; 82(1):1-9.
16. Srikumar R, Parthasarathy NJ, Sheela DR. Immunomodulatory activity of triphala on neutrophil functions. Biol Pharm Bull. 2005; 28(8):1398-403.
17. Perianayagam JB, Sharma SK, Joseph A, Christina AJ. Evaluation of anti-pyretic and analgesic activity of *Emblica officinalis*. Gaertn. J Ethnopharmacol. 2004; 95(1):83-5.
18. Kaur S, Michael H, Arora S, Härkönen PL, Kumar S. The *in vitro* cytotoxic and apoptotic activity of Triphala--an Indian herbal drug. J Ethnopharmacol. 2005; 97(1):15-20.
19. Saeed S, Tariq P. Antibacterial activities of Emblica officinalis and Coriandrum sativum against Gram negative urinary pathogens. Pak J Pharm Sci. 2007; 20(1):32-5.
20. Al Rehaily AJ, Al Howiriny TA, Al Sohaibani MO, Rafatullah S. Gastroprotective effects of 'Amla' *Emblica officinalis*. On *in vivo* test models in rats. 2005; 9(6):515-22.
21. Jain R, Pandey R, Mahant RN, Rathore DS. A review on medicinal importance of *Emblica officinalis*. IJPSR. 2015; 6(1):72-84.
22. Benet LZ, Hosey CM, Ursu O, Oprea TI. BDDCS, the Rule of 5 and drugability. Adv Drug Deliv Rev. 2016; 101:89-98.
23. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Deliv Rev. 2001; 46 (1–3):3-26.

24. Lipinski CA. Lead- and drug-like compounds: the rule-of-five revolution. Drug Discovery Today: Technologies. 2004; 1(4):337-341.
25. Jarrahpour A, Fathi J, Mimouni M, Hadda TB, Sheikh J, Chohan Z *et al*. Petra, Osiris and Molinspiration (POM) together as a successful support in drug design: antibacterial activity and biopharmaceutical characterization of some azo Schiff bases. Medicinal Chemistry Research. 2012; 1(8):1984-90.
26. Suryawanshi SS, Patil RS, Jayannache PB, Palled MS, Alegaon SG, Zaranappa. Screening and assessment of selected alkaloids as potential inhibitors of COVID-19 protease enzyme. Journal of Global Trends in Pharmaceutical Sciences. 2020; 11(2):7711-7718.
27. Jain PK, Joshi H. Coumarin: Chemical and pharmacological profile. J Appl Pharm Sci. 2012; 2(6):236-40.