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Physico-phytochemical and drug likeness profile of fruits of *Capsicum annuum* linn and evaluation of its *In-Vitro* antioxidant activity

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Abstract
The aim of the present research work is to determine the physicochemical, phytochemical and drug likeness profile of selected phytoconstituents present in *Capsicum annuum* Linn and to evaluate the antioxidant activity of ethanolic fruit extract. Drug likeness profile of Quercetin, Luteolin, Beta Carotene, Ascorbic acid, Vitamin A, Vitamin B1, Vitamin B2, Vitamin B3, Vitamin B5, Vitamin B6, Vitamin E and Vitamin K were determined by using online server of Molsoft. Physicochemical properties of powdered material were determined as per standard procedure. Extraction of phytoconstituents from fruits were carried out first with cold maceration (70% v/v ethanol) and then by hot Soxhlet extraction (95% v/v ethanol). Qualitative chemical tests were performed for the detection of primary and secondary metabolites and antioxidant activity was evaluated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method using Quercetin as standard. Results of the study reported the drug likeness profile of phytoconstituents. All the parameters of physicochemical properties were within the acceptance range. Ethanolic extract showed the presence of Alkaloids, Carbohydrates, Glycosides, Saponins, Flavonoids and Tannins. The mean percentage inhibition of DPPH was found to be 247.07% and 363.3% respectively for extract and standard. The IC_{50} values of extract and standard were found to be 1.26µg/ml and 1.11µg/ml respectively.

Keywords: drug likeness profile, antioxidant activity, quercetin, soxhlet extraction, IC_{50}

1. Introduction
In recent days Medicinal plants are in demand and their acceptance is increasing because plants play an important role in ecosystems. Herbs have been considered by human beings since ancient times and these have been used in oldest sciences of various countries such as Egypt, China, Greece, and India. The parts of medicinal plants that may be used are different types of seeds, root, leaf, fruit, flowers or even the whole plant. The active compounds in most parts of the medicinal plants have direct or indirect therapeutic effects and are used as medicinal agents [1]. Phytochemicals have been considered to be of important nutritional compounds to prevent chronic diseases [2].
The Lipinskies rule of five is also known as Pfizer's rule of five or the rule of five (RO5) which is useful for defining the drug like properties of molecules. 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 [3]. The rule is important to keep in mind during drug discovery when a pharmacologically active lead molecule optimization to increase the activity. The drug candidates which obey to the RO5 tend to have lower attrition rates during clinical trials and hence have an increased chance of reaching the market. Knowledge about the drug like properties of molecules plays an important role in the drug discovery [4, 5].
Antioxidants are the molecules that prevent oxidative damage by inhibitingfree radicals which are responsible for producing various disorders in humans like arthritis, ischemia atherosclerosis, and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS. Many synthetic antioxidants cause serious health effects. Recently there is increased demand of interest in the therapeutic potentials of medicinal plants as antioxidants. It has reported that the antioxidant activity of plants might be due to their phenolic compounds. Many plant species have been investigated in the search for novel antioxidants but generally there is still a demand to find more information concerning the antioxidant potential of plant species [6].
Recently, fruits and vegetables have been recognized as natural sources of various bioactive compounds. The main phytochemicals present in the fruits and vegetables are flavonoids, anthocyanins, vitamin c, vitamin E, phenolic compounds, dietary fibres, and carotenoids. Epidemiological studies showed that antioxidant compounds present in the fruits and vegetables can terminate free radical chain reaction. Therefore, it can prevent diseases such as cardiovascular, disease, cancer, diabetes mellitus and liver disorder [7]. One of such vegetable where variety of antioxidants were found is the *Capsicum annuum* Linn. belonging to Solanaceae family and have selected for the present investigation (Figure 1)

Fig 1: Fruits of *Capsicum annum* Linn

The fruits are rich in lipids, carbohydrates, fibers, mineral salts, Vitamin A, B1, B2, B3, B5, B6, C, E and vitamin K, carotenoids, luteolin, Quercetin and beta carotene. Presence of wide range of phytochemicals present in it they are reported to have diverse biological activity. Due to presence of more phenolic content they exhibit good antioxidant activity [8].

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 activity of ethanolic fruits extract. The study also reported the physicochemical and phytochemical properties of fruits.

2. Materials and Methods

2.1 Chemicals and Reagents

All the chemicals and reagents used for study were pure and AR grade and they were collected from store room of KLE's College of Pharmacy, Belagavi. Ethanol for extraction was purchased from Merk.

2.2 Instruments and Apparatus

Hot air oven was used to dry the fruits of capsicum annum, Calibrated weighing balance was used to weigh the crude powder and extract, Muffle furnace was used to carry out the ash value of powdered drug, Soxhlet extractor was used to carry out the extraction, Rota evaporator was used to concentrate the extract.

2.3 Determination of Drug Like Profile of Selected Phytoconstituents

Online web server MolSoft was used for prediction of drug-likeness properties of Quercetin, Luteolin, Beta Carotene, Ascorbic acid, Vitamin A, Vitamin B1, Vitamin B2, Vitamin B3, Vitamin B5, Vitamin B6, Vitamin E, Vitamin K**.** Drug-like properties were calculated based on the Lipinski rule of five. The Canonical simplified molecular line-entry systems (SMILES) were taken from PubChem and subjected to MolSoft web server to determine drug-like profile of molecules [9].

2.4 Collection of plant material

Capsicum annuum Linn. Fruits were collected from the Kallol village of Chikodi Taluk, Belagavi and the plant specimen was subjected for authenticationin Regional Medical Research Centre, Belagavi.

2.5 Drying of fruits and preparation of coarse powder

After authentication crude drug was shade dried and grinded to get coarse powder. Powdered material of fruit was stored in well closed container and protected from light and used for further studies.

2.6 Determination of physical constants of powdered material

Physical constants such as ash values, loss on drying and extractive values of powdered fruit material were performed asper standard procedures [10].

2.6.1. Determination of Ash Values

The total ash, acid insoluble ash and water-soluble ash values were determined for air-dried samples as per the standard procedures. In order to determine total ash value about 2 gm of powdered drug was weighed accurately into a tared silica crucible and incinerated at 450° C in muffle furnace until free from carbon. The crucible was cooled and weighed. Percentage of total ash was calculated with reference to air-dried substance. In order to determine the acid insoluble ash value ash obtained from total ash was boiled with 25 ml of 2 N HCl for few minutes and filtered through an ashless filter paper. The filter paper was transferred into a tared silica crucible and incinerated at 450°C in muffle furnace until free from carbon. The crucible was cooled and weighed. Percentage of acid insoluble ash was calculated with reference to air-dried substance. In order to determine the water-soluble ash value ash obtained from total ash was boiled with 25 ml of distilled water for few minutes and filtered through an ashless filter paper. The filter paper was transferred into a tared silica crucible and incinerated at 450°C in muffle furnace until free from carbon. The crucible was cooled and weighed.

2.6.2. Determination of Loss on drying

The percentages of active chemical constituents in crude drugs are given in terms of air-dried drugs. Hence the moisture content of a drug was determined. In order to determine loss on drying 2 gm of powdered drug was transferred into a Petri dish and the contents were distributed evenly and initial weight was taken. The sample was heated at 105^0C in an oven and weighed. This procedure was repeated until a constant weight was obtained. The moisture content of the sample was calculated with reference to air-dried drug using the following formula.

2.6.3. Determination of Extractive Values

This parameter determines the amount of soluble matter present in the plant. Water, alcohol and ether soluble extractive values were determined as per standard procedure. In order to determine water soluble extractive value 5 g of the crude powder was taken into a conical flask and 100 ml of water was added. This mixture was stirred gently and warmed in a water bath for 30 minutes. The solution was shaken gently at intervals. Then the solution was taken from the water bath and cooled and filtered through a cotton plug. 25 ml of the filtrate was taken and evaporated to dryness. The residue was weighed. Same way in order to determine the alcohol soluble extractives, 5 g of the crude powder was taken in a stoppered flask and 100 ml of ethanol was added. It was shaken continuously for 4 hr on a magnetic stirrer. Then it was filtered by rapidly taking precautions against loss of the solvent. 25 ml of filtrate was evaporated to dryness in a tarred flat-bottomed petri dish, dried at 105°C and weighed. The percentage of ethanol soluble extractive was calculated with reference to air-dried drug. In order to determine the ether soluble extractive, 5 g of the crude powder was taken in a stoppered flask and100 ml of ether was added. It was shaken continuously for 4 hr on a magnetic stirrer. Then it was filtered by rapidly taking precautions against loss of the solvent. 25 ml of filtrate was evaporated to dryness in a tarred flat-bottomed petri dish, dried at 105°C and weighed. The percentage of ethanol soluble extractive was calculated with reference to air-dried drug.

2.7 Preparation of plant extract

50 gm of powder was subjected to maceration with ethanol (70% v/v) for 24 hrs. The marc was then subjected to

Soxhlet extraction with ethanol (95% v/v). Both the extracts were combined and solvent was evaporated in Rota evaporator at 40^0C. Extract was dried and kept in well closed container and protected from light and used for further study [11].

2.8 Qualitative chemical tests
Ethanolic extract was subjected for various phytochemical tests for the detection of alkaloids, glycosides, tannins, flavonoids, steroids, triterpenoids, and saponins. All the tests were performed as per standard procedures [12].

2.9 Evaluation of antioxidant potentials
In this study the antioxidant properties of crude ethanolic extract of *Capsicum annuum* was detected by using DPPH (1,1-diphenyl -2-picrylhydrazyl) radical scavenging assay. This method is based on reduction reaction of alcoholic DPPH with hydrogen donating molecule of antioxidant sample and shows strong absorption band at 517 nm and appears as deep violet colour. The remaining DPPH molecule after certain time, corresponds the radical scavenging activity of antioxidant sample [13].

3. Results and Discussion
3.1. Drug Likeness Properties of Selected Phytoconstituents
The drug like properties and score of Quercetin, Luteolin, Beta Carotene, Ascorbic acid, Vitamin A, Vitamin B1, Vitamin B2, Vitamin B3, Vitamin B5, Vitamin B6, Vitamin E and Vitamin K were presented in Table 1.

Table 1: Drug likeness profile of selected phytoconstituents

Sr. No	Compound Name	Molecular Weight (g/mol)	HBA	HBD	Log p	Drug Likeness Score
1	Quercetin	302.23	7	5	2.11	0.93
2	luteolin,	286.05	6	4	2.78	0.38
3	Beta Carotene	536.44	0	0	13.93	0.64
4	Ascorbic acid	176.03	6	4	1.59	0.74
5	Vitamin A,	286.23	1	1	6.09	0.73
6	Vitamin B1	300.08	4	3	0.66	0.84
7	Vitamin B2	376.14	8	5	1.50	0.62
8	Vitamin B3	123.03	3	1	0.51	0.30
9	Vitamin B5	219.11	5	4	1.75	0.62
10	Vitamin B6	169.07	4	3	0.50	0.71
11	Vitamin E	430.38	2	1	10.08	0.48
12	Vitamin K	450.35	2	0	10.74	0.93

In the present study fruits of *Capsicum annuum* Linn. Were evaluated for it's physicochemical, phytochemical and antioxidant aspects which revealed the following data.

3.2. Collection and authentication of *Capsicum annuum* Linn. Plant
The crude drug collected was authenticated by Dr. Harsha Hegde Scientist from RMRC Belagavi. It was identified and stored with specimen of HerbariumNo. RMRC-976.The powdered material of fruits were subjected for physicochemical, phytochemical and antioxidant evaluation.

3.3. Physical constants
Various physiochemical parameters were evaluated for *Capsicum annuum* dried powder as per standard procedures and results were tabulated in Table 2. Total ash, water soluble ash and acid insoluble ash values of powdered material was found to be 9.48%, 0.58% and 7.63% respectively. Alcohol, water and ether soluble extractive values of powdered material were found to be 17.8%, 7.2% and 1.1% respectively. Loss on drying value was found to be 7.55%.

Table 2: Physicochemical parameters of *Capsicum annuum* powder

Parameters	Values (%W/W)
Total ash	9.48
Watersoluble ash	0.58
Acid insoluble ash	7.63
Alcohol soluble extractive value	17.8
Watersoluble extractive value	7.2
Ether soluble extractive value	1.1
Loss on drying	7.55

3.4. Description of *Capsicum annuum* fruit extract
Extraction was carried out by maceration and soxhlet extraction method. Extract obtained was concentrated using Rota evaporator and observed for its color, consistency and % yield. The ethanolic extract was found exist in reddish brown colour solid. The percentage yield of extract was found to be 39.61%.

3.5. Preliminary phytochemical investigation
In order to detect the presence of various phytochemicals group in ethanolic extract it was subjected to qualitative chemical test. The results of qualitative test showed the presence of carbohydrates, glycosides, alkaloids, saponins, tannins, and flavonoids. The results of investigation were presented in Table 3.

Table 3: Phytochemical investigation data by qualitative analysis

Sr. No.	Phytoconstituents	Name of the test	Results
1	Test for Alkaloids	Mayer's test	+Ve
		Wagner'stest	+Ve
		Dragendorf'stest	+Ve
2	Test for Carbohydrates	Molisch's test	+Ve
		Benedict's test	+Ve
		Fehling'stest	+Ve
		Barford's test	+Ve
3	Test for Glycosides	Modified Borntrager's	+Ve
		Legal'sTest	+Ve
		Balget Test	+Ve
4	Test for Proteins And Amino acids	Millon'stest	-Ve
		Biurettest	-Ve
		Ninhydrintest	-Ve
5	Test for saponins	Foam test	+Ve
6	Test for flavonoids	Shinoda test	+Ve
7	Test for Tannins	$FeCl_3$ test	+Ve
8	Test for Steroids	Salkowski test	-Ve

3.6. Antioxidant potentials of ethanolic extract
The anti-oxidant evaluation of ethanolic extract of fruit was reported in Table 4 and also outcome of the results were showed in Figure 2, 3, 4 and 5. The mean % inhibition of 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml and 25 µg/ml of ethanolic extract were found to be 38.23%, 41.17%,45.32%, 54.12% and 68.23% respectively. The % inhibition produced by standard concentrations of 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml and 25 µg/ml were given as 45.31%, 61.57%, 73.15%, 87.16% and 96.11%respectively. The IC

$_{50}$ values of standard and extract was found to be 1.111 and 1.268 µg/ml respectively.

Table 4: Antioxidant profile of standard and ethanolic extract

Sr. No.	Concentration (µg/ml)	% Inhibition of standard	%Inhibition of Crude extract
1	5	45.31	38.23
2	10	61.57	41.17
3	15	73.15	45.32
4	20	87.16	54.12
5	25	96.11	68.23
Mean		363.3	247.07
IC_{50} Values		1.111	1.268

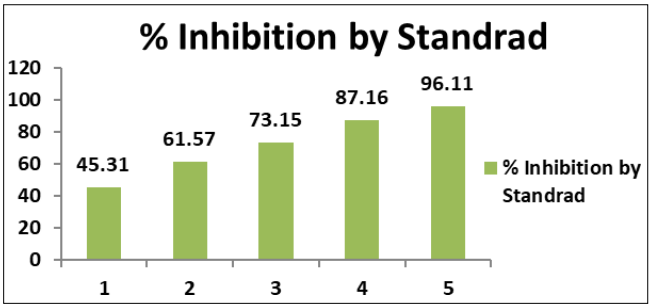

Fig 2: % DPPH Inhibition by Standard

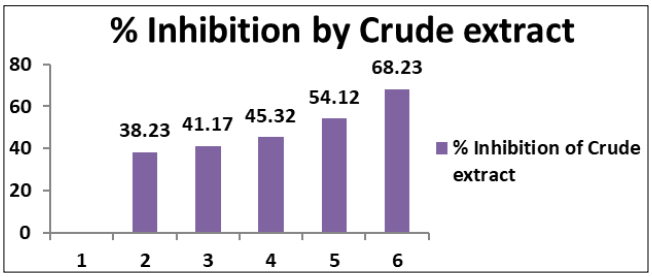

Fig 3: % DPPH Inhibition by Extract

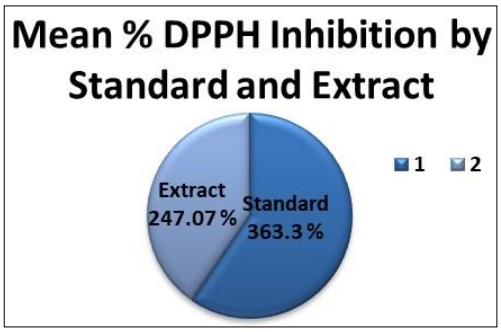

Fig 4: Mean % DPPH Inhibition by Standard and Extract

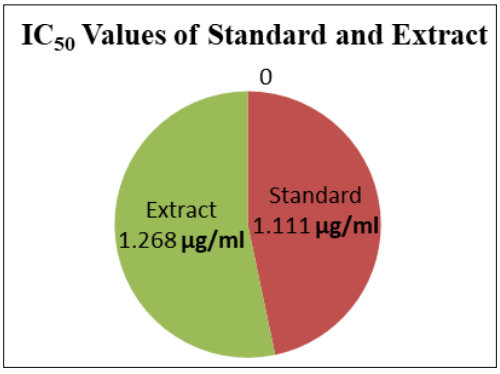

Fig 5: IC $_{50}$ Values of Standard and Extract

4. Conclusion

The present research work concludes that selected phytoconstituents from fruits of *Capsicum annuum* Linn. Showed the good drug likeness score. The ethanolic extract of fruit showed the presence of alkaloids, carbohydrates, glycosides, saponins, flavonoids and tannins. The ethanolic crude extract showed the good antioxidant activity which is compared with standard Quercetin and in future there is need to carry out fractionation and to identify the active fractions.

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6. References

1. Kia1 FJ, Lorigooini Z, Khoei HA. Medicinal plants: Past history and future perspective. Journal of Herbmed Pharmacology. 2018; 7(1):1-7.
2. Sile I, Romane E, Reinsone S, Maurina B, Tirzite D, Dambrova M. Data on medicinal plants in the records of Latvian folk medicine from the 19th century. Data in brief, 2020; 28:1-3.
3. Benet LZ, Hosey CM, UrsuO, Oprea TI. BDDCS, the Rule of 5 and drugability. Adv Drug Deliv Rev, 2016; 101:89-98.
4. 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.
5. Lipinski CA. Lead- and drug-like compounds: the rule-of-five revolution. Drug Discovery Today: Technologies. 2004; 1(4):337-41.
6. Sarkar A, Ghosh U, Natural Antioxidants - The Key to Safe and Sustainable Life. International Journal of Latest Trends in Engineering and Technology. 2016; 6(3):461-6.
7. Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. African Journal of Biotechnology. 2006; 5(11):1142-5.
8. Swamy S, Krupakar A, Surendra CD, Koshy EP. Direct regeneration protocols of five Capsicum annuum L. varieties. African Journal of Biotechnology. 2014; 13(2):307-12.
9. 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):7711-8.
10. Kokate CK. Practical Pharmacognosy. 4th ed. VPBN; New Delhi, 1991.
11. Telagari M, Hullatti K. In-vitro α-amylase and α-glucosidase inhibitory activity of Adiantum caudatum Linn. and Celosia argentea Linn. extracts and fractions. Indian Journal of Pharmacology. 2015; 47(4):425-9.

12. Khandelwal KR, Sethi V. Practical Pharmacognosy Techniques and Experiments. 28 th ed. Nirali Prakashan; Pune, 2017.
13. Aqil F, Ahmad I, Mehmood Z. Antioxidant and Free Radical Scavenging Properties of Twelve Traditionally Used Indian Medicinal Plants. Turk Journal of Biology, 2006; 30:177-83.
14. Zinhoum RA, Negm AA, Abd-El Hameed SM, Afify A. Efficiency of some safe methods in controlling Stegobium paniceum on capsicum minimum plant. International Journal of Entomology Research. 2019;4:07-14.