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Evaluation of Antioxidant and Antiproliferatory Activity of Syzygium Cumini Root Extracts for Possible Anticarcinogenic Potentials

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Research Article

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ABSTRACT

Syzygium cumini (Eugenia jambolana) commonly known as Jamun belongs to the Family Myrtaceae, which is native to India is the folk medicine used in Indian Systems of Medicine from ancient India. The seeds were used in the treatment of Diabetes in the form of Kashaya in Indian Systems of Medicine as well as roots of *Syzygium cumini* were used as folk remedy for Epilepsy. It has been proved that Fruits, Pulp, Stem-bark, Leaves and seeds possess anti-diabetic, anti-inflammatory, Hepato-protective, anti-neoplastic and anti-oxidant activity. This study was performed to evaluate the antioxidant property and anti-proliferatory activity of *Syzygium cumini* root which has been rarely used in the study. Roots of *Syzygium cumini* were collected, washed, shade dried and coarsely powdered. The pharmacognostic assessment was performed systematically and proximate studies reported as total ash 5.5%w/w, Water and Alcohol soluble extractive values 7.42% and 12.3% respectively. The powdered roots were subjected to Continuous hot maceration method using Petroleum ether, Ethyl acetate and Methanol for the extraction of phytoconstituents. These extracts showed the presence of phytosterols, phenolic compounds such as tannins and flavonoids when investigated for phytochemical screening. As methanolic extract showed the presence of phenolic compounds it was further investigated and Total Phenolic Content present in the methanolic extract was expressed as 54.223mg GAE/gm. of extract. DPPH radical scavenging activity was employed in the determination of anti-oxidant activity of Ethyl acetate and methanol extracts .The Standard (L-Ascorbic acid), Ethyl acetate and Methanol extracts were taken in different concentrations ranging from 100µg/ml - 300µg/ml to investigate the percentage of inhibition. The IC50 value of standard, Methanolic extract and ethyl acetate extract was found to be 0.0052µg/ml, 0.00608µg/ml and 3.0801µg/ml. Methanolic extract showed the potent anti-oxidant activity when compared to ethyl acetate extract. MTT assay on A431 Cell Line Culture was considered for the determination of anti-proliferatory property of *Syzygium cumini* root extracts. Doxorubicin was used as standard. Methanol and Ethyl acetate extracts were evaluated at various concentrations ranging from 10µg/ml -320µg/ml. The IC50 Value for Standard Doxorubicin, Ethyl acetate and Methanol extract was reported as 18.47µg/ml, 127.20µg/ml and 143.30µg/ml. Both Ethyl acetate and Methanol extracts have showed the potent anti-proliferatory activity.

Introduction

Around 1350 species of flowering plants and fruit trees belong to this area which includes 717 genera and 160 families, 163 cultivated and 747 medicinal plant species which are used in Ayurveda and Siddha systems of Medicine [1]. The wild edible Species includes fruits, tubers and vegetables etc., which are divided into evergreen and moist deciduous vegetation which influences the growth of the species **(Figure 1)**. The wild fruit species of this area normally comes under both the vegetation's

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were the Flowering and fruiting season lies from February to September and solitary throughout the year. Wild fruit species such as Arthocarpus hirsutus (Jackfruit), *Olea dioica* (Rose Sandalwood), Syzygium caryophyllatum (Wild black Plum), *Chrysophyllum roxburghii*(Indian Star Apple), *Spondias pinnata*(Wild Mango), *Canthium dicoccum*(Ceylon boxwood), *Flacourtia Montana*(Mountain Sweet Thorn), *Mimusops elengi*(Spanish Cherry), *Garcinia gummi-gutta*(Pot Tamarind) comes under Evergreen vegetation, *Grewia tilifolia*(Dhaman), *Phylanthus emblica*(Indian Gooseberry), *Myena laxiflora*(Muyna), *Gmelina arborea*(Beechwood), *Flacourtia indica*(Batoka Plum), *Randia spinosa*(Madanphala), *Careya arboea*(Wild Guava), *Cordia dichotoma* (Indian Cherry), *Terminalia Chebula* (Myrobalan), *Annona reticulate*(Custard Apple) belongs to Moist deciduous vegetation, Whereas Terminalia bellirica(Bahera), Mangifera indica(Mango) and *Syzygium cumini*(Jamun) are dominant in both vegetations[2,3]. Many other wild fruits species are very abundant and have occupied major part of the area. These wild species have reported many medicinally active constituents which play an important role in the treatment of different diseases in local tribal community as well as Indian Systems of Medicine. Many fruits are consumed fresh, some as juice and wine, few are cooked and pickled. Traditionally these fruits are used in the treatment of microbial infections, rheumatism, piles, used in the treatment of Liver related disorders and chronic diseases. These are also used in the treatment of Cancer, treatment of indigestion. It has been reported that it has anti-oxidant, anti-scorbutic, ant-helminthic, anti-inflammatory, anti-neoplastic properties [4]. The major nutritive value of wild fruits are Moisture content (3.60-92.43%), primary metabolites such as Proteins (0.28-17.11%), Carbohydrates (8.62-45.7%), fat (0.17-38.00%) and fiber (0.9-66.56%). Secondary metabolites such as Phenolic(5.31- 49.21mg/g), Flavonoids(0.44-32.12mg/g) are rich , Ascorbic acid(0.01-53.44mg/g), hydroxycitric acid etc., Minerals include Calcium, Magnesium, Manganese, Phosphorus, Zinc, Sodium, Potassium, Nitrogen, Aluminum, Barium, Iron and Copper [5].

According to the research, Head and neck cancers are most prominent cancer affected in India every year. About 10.4% of people of all ages are affected by oral squamous cell carcinoma. Oral cancer is the second largest cancer in India. Many therapies has been established to cure the disease, even Indian systems of medicine also contribute to the treatment.

Syzygium cumini is the well-known plant in the field of Ayurveda from the ancient era. The bark was used to relieve the disorders of gums and teeth. *Syzygium cumini* is rich in compounds containing anthocyanins, glucoside, ellagic acid, isoquercetin, kaempferol and myricetin. The seeds are claimed to contain alkaloid jambosine and glycoside-jambolin or antimellin, which halts the diastatic conversion of starch into sugar and seed extract has lowered blood pressure by 34.6% [6,7]. Various parts of *Syzygium cumini* such as bark, fruit, seed and leaves were used for the treatment of various diseases. The greatest amount of anthocyanins and flavonoids leads to more powerful free radical scavenging effect and there by inhibiting the cancer promoting activity as shown by methanolic extract of *Syzygium cumini*. Scientific studies on *Syzygium cumini* have shown that the various extracts of seed possess a range of pharmacological properties such as antibacterial, antifungal, antiviral, anti-inflammatory, anti-ulcerogenic, cardio protective, anti-allergic, anticancer, radio protective, antioxidant, and hepatoprotective, anti-diarrheal and anti-diabetic effects [8]. Vast number of references revealed that the extracts of different parts of *Syzygium cumini* showed significant pharmacological actions and also found a few significant reports on pharmacological potentials of roots of the plant **(Figure 2)**. But there are no reports pertaining to anti-proliferative activity of *Syzygium cumini* roots. The present study has been undertaken to explore the possible anti-cancer potential of *Syzygium cumini* roots against A431 cell line culture.

Materials and methods

Plant material and preparation

The fresh roots of Syzygium cumini were collected from Madikeri, Kodagu District, and Karnataka. Identification and authentication of the plant material was done by Dr. B K Bharali and Dr. V Rama Rao at Regional Ayurveda Research Institute for Metabolic Disorders.

40 Gms of dried coarsely powdered *Syzygium cumini* roots were packed and loaded in syphon tube using thimble each time for soxhlation **(Figure 3)**. Petroleum ether, Ethyl acetate and Methanol used as solvents in increasing order of polarity at 40oC. Extraction of constituents using each solvent was carried out for 8 hrs. Until syphon tube was colorless. The marc was dried before treating with individual solvent, the individual solvent extract was concentrated using Rota-evaporator and combined. The percentage yield was calculated on basis of air dried weight of the plant.

Figure 1. Photograph representing *Syzygium cumini* tree and fruits.

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Figure 2. Photograph representing *Syzygium cumini* tree and Roots.
Wild *Syzygium cumini* tree, (b) *Syzygium cumini* tree from where the roots were uprooted

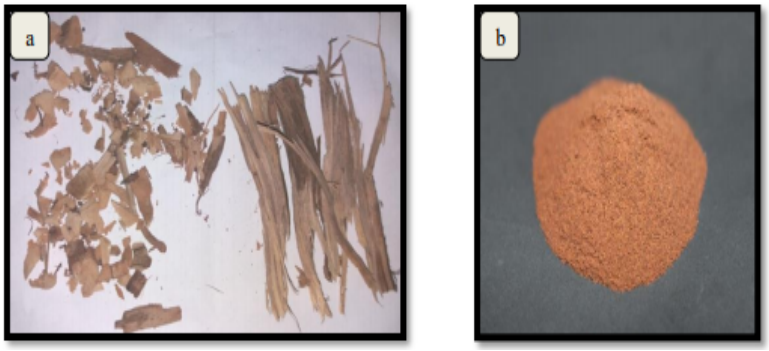

Figure 3. Photograph representing Types of *Syzygium cumini* Roots.
Wild *Syzygium cumini* tree, (b) *Syzygium cumini* tree from where the roots were uprooted

Results

Pharmacognostical Evaluation of Syzygium Cumini roots as per WHO guidelines

Phytochemical Details

Phytochemical screening of *Syzygium cumini* extracts in different solvents was carried out and result found that Glycosides, phenols, proteins, steroids and tannins were detected in the acetone extract. Alkaloids, proteins and steroids from chloroform extract. Alkaloids, carbohydrates, flavonoids, glycosides, phenols, resins, saponins and tannins from the methanol extract and only alkaloids were detected from the n-hexane extract. The stem bark of *Syzygium cumini* showed the presence of flavonoids, glycosides, phenol, proteins, resins and saponins in the acetone extract. Alkaloids, carbohydrates, flavonoids, glycosides, phenols, resins, saponins, steroids and tannins were recorded in the methanol extract. Only alkaloids and tannins were detected in the chloroform extract whereas alkaloids, proteins and tannins were detected in the n-hexane extract. In the root extract all the tested phytoconstituents were detected in the methanol extract. Alkaloids, carbohydrates, phenol, proteins and tannins were detected in the acetone and chloroform extracts, but only carbohydrates and proteins were detected from n-hexane extract. Screening of seed extracts of S.cumini showed that alkaloids, flavonoids, phenols, proteins, resins and tannins were detected in methanol extract, alkaloids, carbohydrates, phenols, proteins and tannins were found in the acetone and chloroform extracts whereas only carbohydrates and proteins were detected in the n-hexane extracts.

HPTLC analysis

The presence of various phytoconstituents from the ethanol and aqueous extracts of leaf, stem bark, flower, root and seeds of *Syzygium cumini* was determined by phytochemical screening and HPTLC analysis. The plant extract contains carbohydrates, proteins, alkaloids, flavonoids, tannins, steroids, tri-terpenoids, phenol and saponins. The study provided evidence that the plant contains medicinally bioactive compounds and this justifies the use of the plant species as traditional medicine for treatment of diabetes.

HPLC-DAD-MS/MS

The composition of carotenoids and phenolic compounds from jambolao fruits (*Syzygium cumini*) was investigated by HPLC-DAD-MS/MS. Two main carotenoids were found in the fruits, all-trans-lutein (43.7%) and all-trans-b-carotene (25.4%). The anthocyanin composition was characterized by the presence of 3, 5-diglucosides of five out of six aglycones commonly found in foods. This pattern was also observed for the other flavonoids, since diglucosides of dihydromyricetin, methyl-dihydromyricetin and dimethyldihydromyricetin, along with myricetin glucoside and a galloyl glucose ester were identified.

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Extraction of Syzygium Cumini Roots

40gms of each powdered drug was extracted individually with petroleum ether, ethyl acetate and methanol with increasing polarity by soxhlation **(Figure 4)**. Before extracting with next solvent the marc was pressed to remove the residual solvent, combined and concentrated to get the concentrated extract. The percentage yield was calculated with respect to dry weight of the drug, Color and consistency of the extracts were noted. Petroleum ether extract was Buff color, oily in consistency with 0.75% yield. Ethyl acetate extract was Pale yellow, powder in consistency with 1.36% yield. Methanol extract was dark brown crystals with 13.22% yield.

Proximate Analysis

The proximate analysis determines the percentage of foreign particles present in the drug and authenticity of the selected material with respect to the literature survey. The proximate analysis of the involved the total ash, alcohol extractive value and water extractive values which are found to be 5.525 ± 0.012, 12.36 ± 0.53 and 7.42 ± 0.52 respectively. The observations are noted in **(Table 1).**

Determination of Total Phenolic Content

Phenolic compounds are very abundant in natural plants. This includes Gallic acid, ellagic acid, Resveratrol, Caffeic acid, Ferulic acid, Kaempferol, Myricetin, anthocyanidin and many more. The estimation of the phenolic compounds is necessary to determine the amount of phenolic compound present in the selected plant material. Phenolic compounds are estimated and represented as Gallic acid Equivalent, as Gallic acid is used a Standard phenolic compound. The percentage of Phenolic compound present in the methanolic extract of Syzygium cumini roots were determined by Folin-Ciocalteu method. The calibration curve was plotted using Gallic acid as standard. TPC present in methanolic extract was expressed as 54.22mg Gallic Acid Equivalent/ gm. of extract.

Pharmacognostic evaluation

The selected plant material has been subjected to systematic Pharmacognostical evaluation to identify its different specifications such as macroscopical and microscopical characters which gives clear information of the plant. It also involves the proximate analysis which includes determination of Ash value and Extractive values. This standardized evaluation has carried out with respect to WHO guidelines.

Organoleptic Characteristics of *Syzygium cumini* roots. The selected *Syzygium cumini* roots were subjected to macroscopic evaluation by determination of the size, color, odor, taste and shape of the drug. The observations are noted in **(Table 2).**

Evaluation of In-Vitro Anti-Oxidant Activity of Syzygium Cumini Root Extracts

DPPH radical scavenging assay method was incorporated in the determination of anti-oxidant activity of Syzygium cumini roots extracts. Ascorbic acid was used as positive control. All the samples showed percentage of inhibition at different concentrations. The IC_{50} value of Standard ascorbic acid was found to be 0.0053µg/ml the lowest IC_{50} value found was methanolic extract followed by ethyl acetate extract with 0.0068µg/ml and 3.0801µg/ml. The results are summarized in **(Table 3, Figure 5 and 6).**

Evaluation of In-Vitro Anti-Proliferatory Activity of Syzygium Cumini Root Extracts.

In determination of Anti-proliferatory activity of *Syzygium cumini* roots, Ethyl acetate and methanol extracts of roots were tested on A431 (Epidermal Carcinoma Cell line). Based on MTT assay, the viable cells were calculated. Doxorubicin was used as positive control. The different sample concentrations were taken from 10µg/ml to 320µg/ml. The graph was plotted and IC_{50} value of methanol and Ethyl acetate extract was 127.20µg/ml and 143.30µg/ml. The results are summarized and represented in **(Table 4 and 5, Figures 7 and 8)**

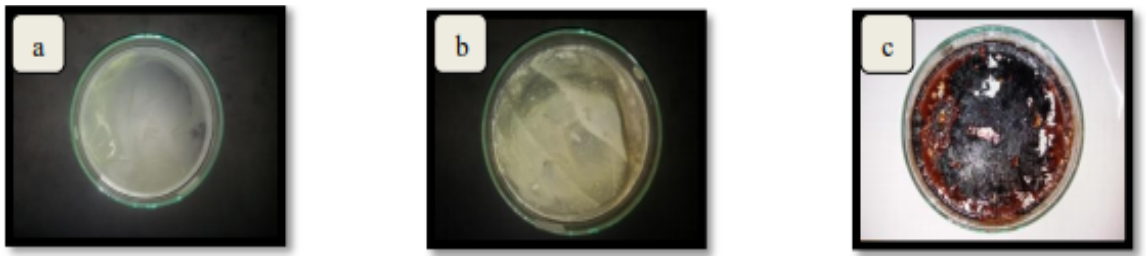

Figure 4. Extraction of *Syzygium Cumini* Roots.
(a) Petroleum ether extract, (b) Ethyl acetate extract, (c) Methanol extract of Syzygium cumini roots

Table 1:- Proximate Analysis of *Syzygium cumini* root powder.

S No.	Standard Parameters (%)	Syzygium cumini
1	Total Ash Value	5.525 ± 0.012
2	Alcohol Extractive Value	12.36 ± 0.53
3	Water Extractive Value	7.42 ± 0.52

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Table 2: Macroscopic characteristics of *Syzygium cumini* root

S No.	Characteristics	Syzygium cumini roots
1	Size and Shape	Root consists of reddish pink bark with smooth surface and buff color heart wood. 8.0cm in diameter and root bark is of 0.1 -0.2 mm thickness with fibrous fracture.
2	Color	Reddish pink bark, buff colored heart wood.
3	Taste	None
4	Odor	Characteristic

Table 3:- Percentage inhibition of Different extracts of *Syzygium cumini* roots.

S no.	Concentration (µg/ml)	Ethyl Acetate Extract	Methanol Extract	Ascorbic Acid (Positive control)
1	100µg/ml	19.230 ± 0.021*	88.082 ± 0.052*	96.336 ± 0.056*
2	150µg/ml	38.278 ± 0.014*	88.341 ± 0.002*	96.420 ± 0.025*
3	200µg/ml	39.926 ± 0.033*	89.637 ± 0.056*	96.531 ± 0.042*
4	250µg/ml	50.000 ± 0.052*	90.932 ± 0.013*	96.621 ± 0.002*
5	300µg/ml	57.326 ± 0.025*	91.191 ± 0.065*	96.703 ± 0.010*

Figure 5. Photographs of in-vitro antioxidant activity of *Syzygium cumini* root extracts

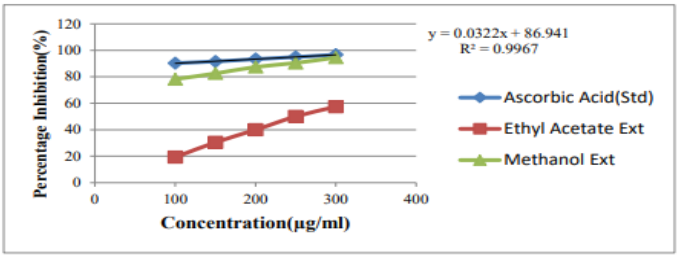

Figure 6. Graph of In-vitro Antioxidant activity of *Syzygium cumini* root extracts.

Table 4:- Anti- Cancer activity of Doxorubicin (Positive Control) on A431 cell line culture.

A431			Standard	
Compound name	**Conc. µM**	**OD at 590nm**	**% inhibition**	**IC50 µM**
Control	0	0.566	0	
	3.12	0.495	12.54	
	6.25	0.418	26.15	
Doxorubicin	12.5	0.341	39.79	18.47
	25	0.239	57.72	
	50	0.151	73.32	
	100	0.079	86.1	

Table 5:- Anti-cancer activity of different extracts *Syzygium cumini* on A431 cell line culture.

A431				
Compound name	**Conc. µg/ml**	**OD at 590nm**	**% Inhibition**	**IC50 µg/ml**
Control	0	0.566	0	
	10	0.475	16.04	
	20	0.402	28.94	
Methanol extract	40	0.368	35.03	
	80	0.317	43.95	
	160	0.25	55.83	127.2
	320	0.155	72.6	
	10	0.466	17.61	
Ethyl acetate extract	20	0.422	25.36	
	40	0.394	30.42	
	80	0.338	40.23	143.3
	160	0.243	57.09	
	320	0.183	67.73	

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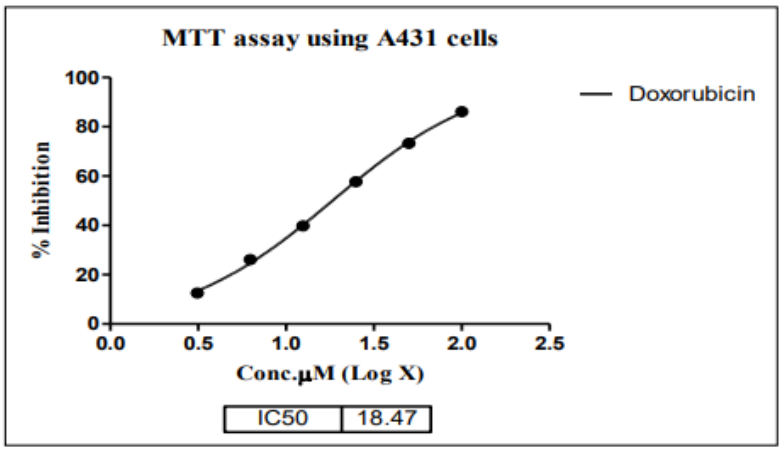

Figure 7. Graph of anti-cancer activity of Doxorubicin (Positive Control) on A431 cell Line culture.

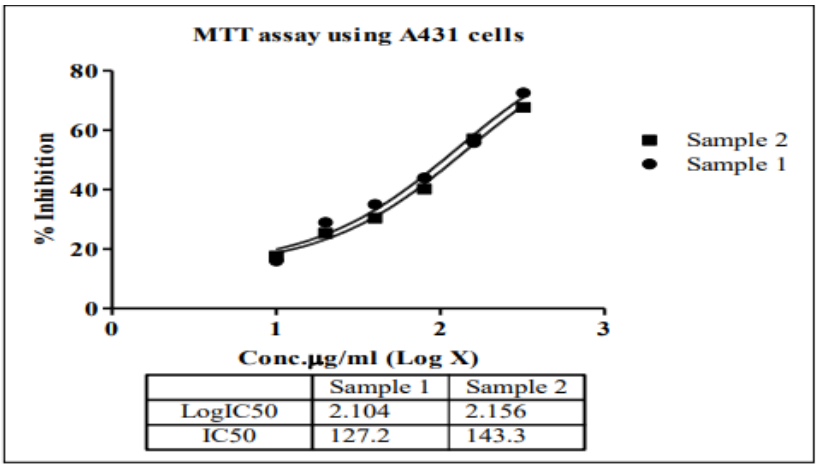

Figure 8. Graph of anti- Cancer activity of Ethyl acetate and Methanol on A431 cell line culture.

Statistical analysis

All data are presented as the mean ± the standard error of the mean. The data were analyzed using Graph Pad Prism Version 6.0 (Graph Pad Software, Inc.) and compared using a one-way ANOVA with a Tukey's multiple comparison test $P<0.05$ was considered to indicate a statistically significant difference.

Discussion

India is well known for its heritage of culture, art and medicine. It stands unique among all the countries and civilizations in world. In spite of the all this, it have greater contribution in the field of medicine. Ayurveda being the oldest systems of medicine in India has its own significance in the treatment of many diseases which have a lower impact by allopathic medicine. Taking into consideration this study was carried out with reference to Ayurvedic System of medicine, where many herbs were used in the treatment. *Syzygium cumini* is wild tropical fruit tree, which is 2500 years native to India. It is being used as the “Folk medicine" in the field of Ayurveda from ancient India, is also called as "Fruit of Gods" due to its importance in Indian Mythology. It was mainly used in the treatment of open wounds, diabetes, treating teeth and gum disease and also used as anthelmintic [9]. Various parts of *Syzygium cumini* has various phytoconstituents which as its own importance in the treatment of diseases. In this Study, mainly roots were considered as the literature survey showed the minimal numbers of references adding to these roots are rich in polyphenols such as flavonoid glycosides and isorhamnetin 3-o-rutinoside [10], which have showed the significant therapeutic action in the treatment of Carcinoma. This study is conducted to demonstrate the therapeutic significance of *Syzygium cumini* roots which are rarely used in the field of research. The macroscopy and microscopy of the roots were carried out to evaluate its identity and authenticity according to the literature survey. The proximity studies of the *Syzygium cumini* roots was conducted to determine the inorganic matter present in the roots the results found were within the limit of the WHO guidelines.

The phytochemical screening of showed the presence of phytosterols and phytopolyphenols in ethyl acetate and methanol extracts. Phytosterols and Phytopolyphenols have capability to inhibit metastasis, differentiation and proliferation of cancer cells [11, 12]. Based on this, it was evaluated for anti-oxidant and anti-proliferatory property. *Syzygium cumini* roots are rich in polyphenols such as flavonoid glycosides and isorhamnetin 3-o-rutinoside [10]. In this study, methanolic extract showed the presence of phenolic compounds, the quantification of the phenolic content was carried out by using F-C reagent method as it contains mixture of phosphomolybdate and phosphotungstate which reacts with the phenolic content present in the drug in the presence of alkaline medium by the transfer of electrons [13]. This is detected by the formation of the blue chromophore due to phosphomolybdate and

phosphotungstate complex, considering the absorbance at 765nm. Then, both methanol and ethyl acetate extracts of *Syzygium cumini* root were subjected to in-vitro antioxidant activity, in which methanolic extract showed significant result than ethyl acetate extract by reducing DPPH stable free radical by donating hydrogen electron which leads to the formation of DPPH-H [14]. This is detected by, the decolorization of the Violet color of DPPH solution to Yellow color in the presence of antioxidant agent. Gallic acid is the standard antioxidant agent used. The maximum absorption was noted at 517nm using UV Spectrophotometer [15] .The IC50 values of standard, methanolic extract and ethyl acetate were determined as 0.0056µg/ml, 0.0068µg/ml and 3.0801µg/ml. The IC50 value of Methanolic extract was nearly to standard, by this we can say that phenolic compounds present in methanolic extract may be responsible for the free radical scavenging activity. Ethyl acetate and methanolic extracts were subjected to anti-proliferatory activity on A431 cell line culture by using MTT Cell Proliferation assay which is a colorimetric assay used in the determination cell viability. MTT 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) is a yellow tetrazolium salt which is reduced by the viable cells by the action of the dehydrogenase enzyme to the formation of NADH and NADPH. This results in the formation of intracellular purple formazon which can be solubilized by DMSO, acidified isopropanol or other solvents and spectrometrically measured. The formazon formation depends on number of viable cells indicating the effects of individual drug on the cells [16]. Both ethyl acetate and methanol extracts of Syzygium cumini roots significant results with IC50 value of 127.2 µg/ml and 143.3µg/ml whereas the standard used Doxorubicin has showed IC50 value of 18.47 µg/ml. So, from the above results we can say that compounds present in ethyl acetate extract have showed better anti-proliferatory activity than methanolic extract compounds. As Ethyl acetate extract showed the presence of Phytosterols, these compounds may be responsible for Cell-proliferation than polyphenols.

Conclusion

The present study showed significant results in analyzing the anti-carcinogenic potential of *Syzygium cumini* roots evaluated by in-vitro anti-oxidant and anti-proliferatory activity. By this we can conclude that Phytopolyphenols and Phytosterols present in *Syzygium cumini* roots are responsible for significant anti-oxidant property and anti- proliferative property.

Acknowledgment

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

POE and WD performed the experiments, analyzed, interpreted the data and wrote the manuscript. WM and IP conceived and designed the study, supervised the study, interpreted the data and drafted the manuscript. ST designed and supervised the study, interpreted the data, discussed the results, wrote and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable

Patient consent for publication

Not applicable.

Conflicts of interest

“There are no conflicts to declare”.

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