# *PIPER BETLE* (L.) CULTIVARS: A POSSIBLE SOLUTION TO MITIGATE ORAL CANCER RISKS IN QUID CHEWERS

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## I. Abstract

*Piper betle*, often referred to as the "Golden Heart of Nature" and the "Green Gold of India," has been extensively utilized for its vast array of therapeutic and medicinal properties. This plant is rich in phytochemical compounds, including tannins, terpenoids, alkaloids, flavonoids, saponins, and phenols. Additionally, it contains essential vitamins such as B, C, and A, along with minerals, proteins, calcium, phosphorus, iron, iodine, potassium, and carbohydrates. The essential oils of P. betle are characterized by constituents like cadinene, carvacrol, allyl catechol, chavicol, p-cymene, caryophyllene, chavibetol, cineole, eugenol, estragole, and more. These elements contribute to its wide-ranging pharmacological benefits, which include anti-allergic, anti-fertility, anti-larvicidal, anti-filarial, anti-cancer, cytotoxic, anti-fungal, anti-nociceptive, insecticidal, anti-halitosis, gastro-protective, anti-diabetic, anti-platelet, anti-dermatophytic, wound healing, antioxidant, immunomodulatory, and antiseptic activities. Despite the wealth of reports on the medicinal properties of P. betle, the variety and regional differences in common names pose challenges in correlating data for specific material. Oral cancer remains a significant concern in Asian countries, primarily due to the prevalent practice of chewing betel quids containing tobacco, coupled with smoking and alcohol consumption. This study aims to identify a novel herbal chemotherapeutic agent by harnessing the antioxidant properties of P. betle, thereby offering additional benefits to quid chewers. Furthermore, this research seeks to highlight the differences among various P. betle varieties such as Calcutta, Maghai, Banarasi, Poona and determine the optimal combination for mitigating the risk of oral cancer.

## II. Introduction

Betel leaf, known scientifically as *Piper betle*, is a highly regarded as commercial botanical with numerous therapeutic properties. This evergreen perennial vine belongs to the Piperaceae family and has been valued for its medicinal, nutritional, and cultural significance for over two millennia (Rintu, et al., 2015). Traditionally, betel leaf has been used as an adjuvant in various medicines and is praised for its diverse therapeutic benefits, including improving voice clarity, acting as a laxative and appetizer, combating bad breath, treating boils, relieving constipation, and addressing conjunctivitis, among other uses (Guha, 2006;

Pradhan, et al., 2013). In Ayurveda, it is known for its ability to suppress vata and kapha doshas, further underscoring its therapeutic versatility (Pradhan, et al., 2013). Betel leaves are also rich in vitamins and minerals, making them highly nutritious (Smila, et al., 2014). According to the Yunani system, betel leaves have a sharp taste and pleasant aroma, enhancing taste and appetite, and serving as an excellent tonic for the brain, heart, and liver (Pradhan, et al., 2013). *P. betle* leaves are renowned for their wide range of medicinal properties, with over 100 distinct varieties found globally (Smila, et al., 2014). The variation in phytoconstituents among these cultivars results in differing medicinal and aromatic qualities. However, much of the existing literature often fails to specify the cultivar type, which is essential for understanding their therapeutic potentials. Chewing betel leaves is a common practice in Asia, known to induce a sense of well-being, heightened alertness, and physiological responses such as increased sweating and salivation. The leaves are aromatic, sharp, and acrid, making them ideal for mastication when combined with substances like *Areca catechu* (betel nut), lime churna, catechu (katha), and sometimes tobacco. While betel leaves are nutritive and stimulating, excessive consumption, particularly when mixed with tobacco, can lead to adverse effects such as dental issues, oral infections, palpitations, and potentially oral cancer (Guha, 2006). Conversely, non-tobacco quids do not cause these harmful effects, though this distinction has been the subject of ongoing controversy. Understanding the specific cultivars and their phytoconstituents is crucial in evaluating their varying therapeutic and health impacts. Betel leaves are recognized for their high content of flavonoids and phenols, which contribute to potent antioxidant, radical scavenging, and anticancer properties (Harini, et al., 2018). These leaves contain various antioxidants such as flavonoids, terpenoids, tannins, alkaloids, and saponins (Singh, et al., 2023). Unlike tobacco containing mixtures, non-tobacco quids involving betel leaves are generally considered safer due to their antioxidant properties. Compounds like hydroxychavicol and chlorogenic acid in betel leaves are known for their ability to combat carcinogens and possess antimutagenic effects, countering the potential harm caused by tobacco (Guha, 2006; Uddin, et al., 2015). Free radicals, which are harmful and linked to serious health conditions such as cancer, cardiovascular diseases, and Alzheimer's disease among others (Bhuvaneswari, et al., 2014; Manigauha, et al., 2009), are effectively neutralized by antioxidants. Natural antioxidants like phenolics and flavonoids

play a crucial role in protecting against oxidative stress by scavenging free radicals and acting as reducing agents, thus proving valuable as alternatives to synthetic antioxidants known for their potential toxicity (Jaiswal, et al., 2014; Venugopalan, et al., 2015). Extensive failures in oral cancer treatments often result in drug toxicity and resistance, highlighting the need for less toxic chemotherapeutic agents. Oral cancer is the sixth most malignant in the world, because of prevalent habit of betel quid chewing, smoking, and alcohol consumption in Southeast Asia (Veettil, et al., 2022). India has one of the highest oral cancer cases globally (Dinesh, et al., 2016). *P. betle*, though a main ingredient of quid it is known to be rich in antioxidant, phenolic and flavonoid compounds, that may aid in this effort. Consuming betel leaves with tobacco might help in aiding to reduce tobacco-related cancer risks, as plant extracts rich in flavonoids and phenolic compounds are potential sources of chemotherapeutic agents (Boontha, et al., 2019). In this study, we investigated the antioxidant activity, total phenolic content, total flavonoid content, and anticancer activity of the leaves from four different *P. betle* cultivars: Calcutta, Maghai, Banarasi, and Poona. This comparative analysis aims to identify which cultivar exhibits superior properties. Understanding these differences will provide insights into their respective strengths and help in developing an optimal combination of chemotherapeutic agents based on the unique properties of each cultivar.

## III. Materials and Methods

Collection and Authentication: Four P. betle varieties (Calcutta, Maghai, Banarasi, Poona) were collected from South Mumbai markets and authenticated at Blatter's Herbarium, St. Xavier's College, Mumbai. Extraction: The collected leaves were washed, dried at 50°C for 3-4 days, and ground into powder. The powder was subjected to cold maceration in ethanol using a shaker apparatus, involving 8 hours of shaking and 16 hours of static extraction (Sankeshwari. et al., 2018).

Determination of Antioxidant potential:

1. DPPH Assay: The antioxidant activity of the extracts was evaluated using the DPPH radical scavenging assay, following the method outlined by Fang et al. (2017). A 0.1 mM DPPH solution was prepared using 50% methanol, into which 2.0 ml of the sample was added. After thorough

mixing, the mixture was incubated for 30 minutes. Ascorbic acid (100 µg/ml) served as the standard antioxidant. The absorbance of the samples was measured at 517 nm using a UV-Vis spectrophotometer (SHIMADZU UV VIS Spectrophotometer Model No 1780), and the percentage of Radical Scavenging Activity (%RSA) was calculated using the formula: %RSA = (Abs of Control - Abs of Test) / Abs of Control × 100.

2. FRAP Assay: The Ferric reducing power of the extracts was determined using a method adapted from Benzie and Strain (1996) with some modifications. The FRAP (Ferric Reducing Antioxidant Power) assay was conducted at low pH using a specially prepared FRAP reagent. This reagent was made by mixing 25 ml of 300 mM acetate buffer (pH 3.6) with 2.5 ml of TPTZ solution in 40 mM HCl and 2.5 ml of 20 mM ferric chloride solution. To test the samples, 100 µl of each extract was added to 3.9 ml of the working FRAP reagent. A standard curve was established using ferrous sulphate solutions ranging from 0.2 to 1.0 mM. The absorbance of the resulting solutions was measured at 593 nm using a UV-Vis spectrophotometer (SHIMADZU UV VIS Spectrophotometer Model No 1780). The results were reported in terms of mM FeSO4 equivalents.

Determination of Total Phenolic and Total Flavonoid Content:

1. Total Phenolic Content The total phenolic content in ethanolic extracts was determined using the Folin-Ciocalteu method as described by Mukherjee (2019) with some modifications. A 0.1 ml portion of the sample was mixed with 0.1 ml of Folin-Ciocalteu phenol reagent, followed by the addition of 1.0 ml of 7% sodium carbonate solution and 3.4 ml of distilled water. After thorough mixing, the mixture was incubated for 30 minutes, and the absorbance was measured at 760 nm using a SHIMADZU UV-VIS Spectrophotometer Model No 1780. A calibration curve was constructed using a standard Gallic acid solution ranging from 0.25 to 0.1 mg/ml. The total phenolic content was expressed as milligrams of Gallic acid equivalents per gram of sample, calculated using the formula $TPC = C \times V / m$, where C is the concentration obtained from the Gallic acid standard curve, V is the volume of the sample used, and m is the mass of the sample.

2. 2 Total Flavonoid Content The total flavonoid content in ethanolic leaf extracts was assessed using the $AlCl_3$ colorimetric method as described by Mukherjee (2019) with minor adjustments. Specifically, 2.0 ml of plant extract was combined with an equal volume of 2% $AlCl_3$ reagent and thoroughly mixed. After a 30-minute incubation period, the absorbance was measured at 415 nm using a UV-Vis spectrophotometer (SHIMADZU UV VIS Spectrophotometer Model No 1780). A calibration curve was constructed using standard Rutin solutions ranging from 0.01 to 0.05 mg/ml. The total flavonoid content was quantified as milligrams Rutin equivalents per gram using the formula $TFC = C \times V / m$, where C represents the concentration determined from the Rutin standard curve, V is the volume of the sample utilized, and m denotes the mass of the sample.

Anti-Cancer activity (Venkanna, et al., 2014)

1. Principle MTT Assay is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol- 2-yl)-2,5- diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The assay depends both on the number of cells present and, on the assumption, that dead cells or their products do not reduce tetrazolium. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, dark purple coloured formazan crystal. The cells are then solubilized with a DMSO and the released, solubilized formazan reagent is measured spectrophotometrically at 570 nm.
2. Chemicals RPMI, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], trypsin, EDTA Phosphate Buffered Saline (PBS) and were purchased from Sigma Chemicals Co. (St. Louis, MO) and Fetal Bovine Serum (FBS) were purchased from Gibco. 25 $cm^2$ and 75 $cm^2$ flask and 96 well plated purchased from Eppendorf India.
3. Maintenance of cell line The cancer cell lines were obtained from NCCS, Pune, and cultured in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% fetal bovine serum (FBS) and 0.5 mL/L penicillin/streptomycin antibiotics. The cells were maintained in a controlled environment at 37°C with 5% $CO_2$ and 95% air. This standard culture condition provided optimal growth and maintenance of the cells for experimental purposes.

4. Preparation of test compound For the MTT assay, the stock extracts were diluted to a final concentration of 1 mg/ml. Cancer cells were then exposed to various concentrations ranging from 5 to 100 µg/ml of these diluted extracts. The MTT assay measures cell viability based on the ability of viable cells to convert the yellow MTT (3-(4,5-dimethylthiazol2-yl)-2,5-diphenyltetrazolium bromide) to a purple formazan product through mitochondrial activity. This range of concentrations allows for the assessment of how the extracts affect cell proliferation or viability in a dose-dependent manner.
5. MTT Assay Cell viability was evaluated by the MTT Assay with five concentrations of extract in triplicates. Cells were trypsinized and preformed the tryphan blue assay to know viable cells in cell suspension. Cells were counted by hemocytometer and seeded at density of 5.0 X 103 cells / well in 100 µl media in 96 well plate culture medium and incubated overnight at 37 0 C. After incubation, taken off the old media and added fresh media 100 µl with different concentrations of test extract in represented wells in 96 plates. After 48 hrs., Discarded the solution and added the fresh media with MTT solution (0.5 mg / mL-1) was added to each well and plates were incubated at 370C for 3 hrs. At the end of incubation time, precipitates are formed as a result of the reduction of the MTT salt to chromophore formazan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was measured at 570 nm on a microplate reader. The percentage growth inhibition was calculated using the following formula such as % Inhibition = 100(Control- Treatment) / Control. The IC50 value was determined by using linear regression equation i.e. $y = mx + c$. Here, $y = 50$, m and c values were derived from the viability graph.

## IV. Result & Discussion

### A. *Antioxidant Activity, Total Phenolic Content and Total Flavonoid Content*

The antioxidant activity of the extracts was calculated in terms of percent DPPH radical scavenging activity and compared with that of Ascorbic acid. %RSA of 100 µg/ml of leaf extract is represented in figure no. 1 and figure no. 2. Free radical reducing power of extracts were calculated from Standard Ferrous sulphate curve ($y=0.1936x$; $R2 = 0.9964$) as in figure no. 1(A). The FRAP value of leaf extracts was reported in terms of mM equivalents of $FeSO4$ in figure no. 1 and figure no. 2. The TPC of the leaf extracts was calculated from standard Gallic Acid curve ($y = 0.3001x$; $R2 = 0.9776$) as shown in figure no. 1(B) and represented the comparison in figure no. 2. The TFC of the leaf extracts was calculated from standard Rutin curve ($y = 18.421x$; $R2 = 0.9974$) as shown in figure no. 1(C) and represented the comparison in figure no. 1 and figure no. 2.

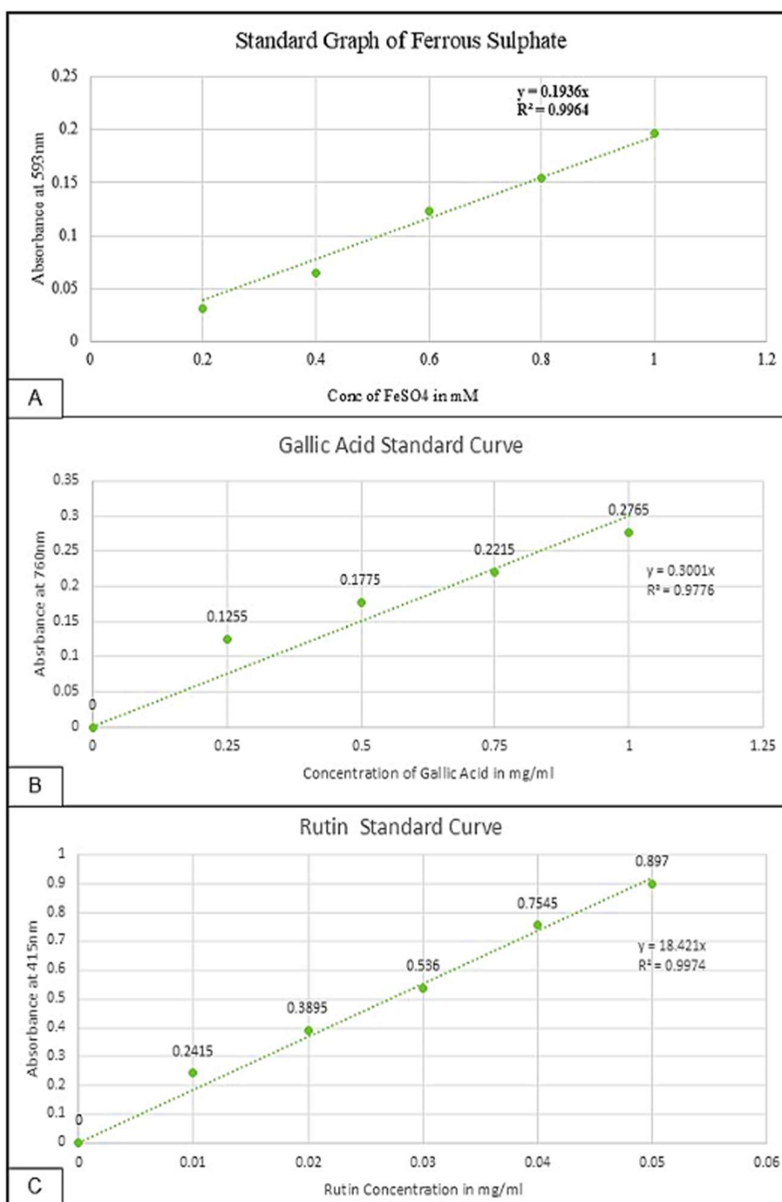


**Fig 1: Standard Graphs for A: FRAP; B: TPC; C: TFC**

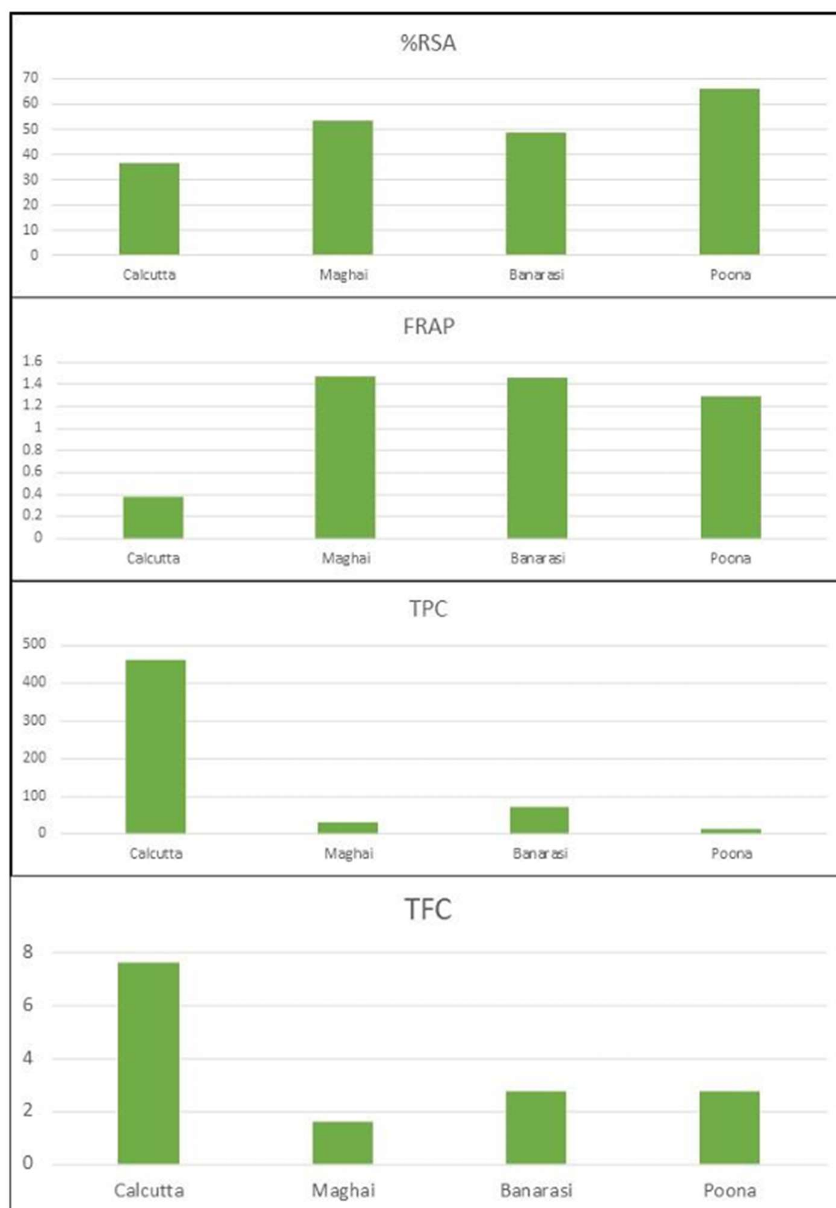


**Fig 2: Comparative Graphs for all the analysis performed**

### *B. Anti-Cancer activity*

The Cytotoxic effect on increasing concentration of *P. betle* (Calcutta, Maghai, Banarasi and Poona) leaf extract of KB Cell lines was represented in figure no. 3 and figure no. 4 and cell viability and percentage viability were shown in table no. 1 respectively. The Percentage Viability graph to determine $IC_{50}$ values for *P. betle* (Calcutta, Maghai, Banarasi and Poona) is shown in figure no. 4.

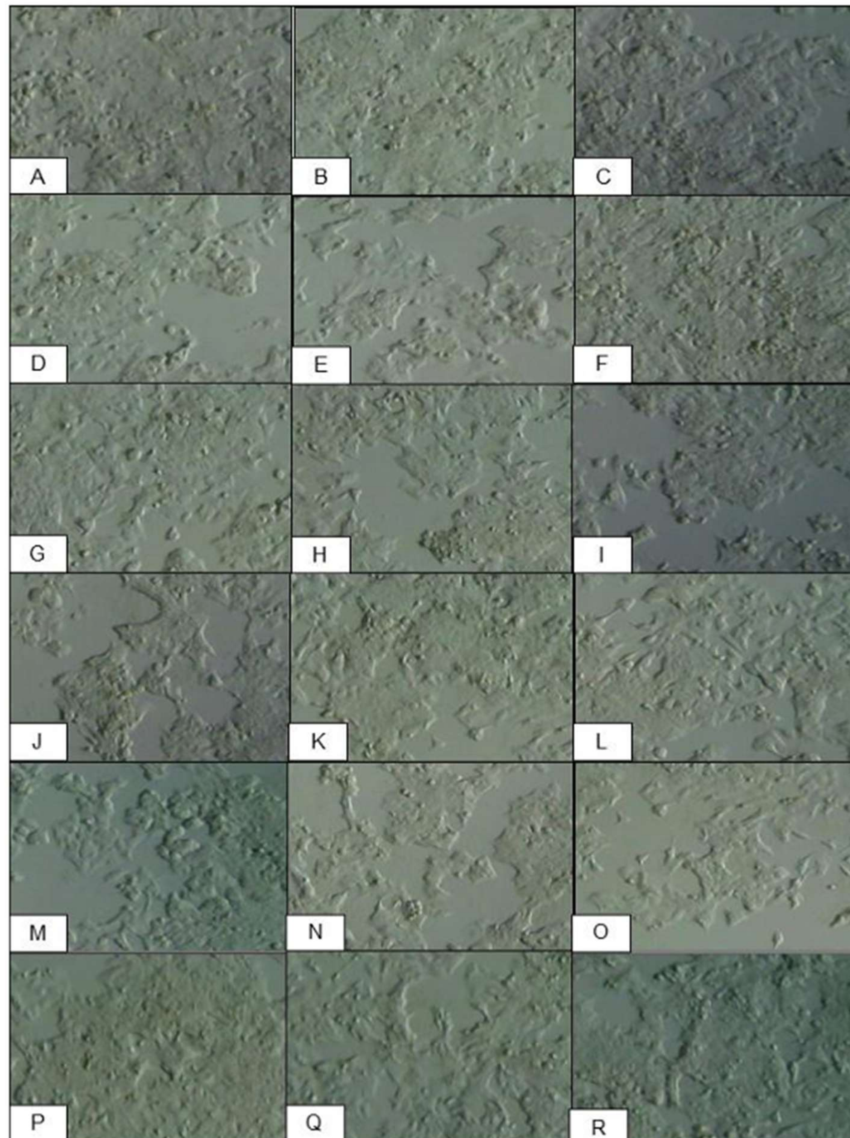


**Fig. 3: Cytotoxic effect on increasing concentration of P. betle leaf extract of KB Cell lines; A: Calcutta-5ug, B: Calcutta-10ug, C: Calcutta-25ug, D: Calcutta-50ug, E: Calcutta-100ug, F: Maghai-5ug, G: Maghai-10ug, H: Maghai-25ug, I: Maghai-50ug, J: Maghai 100ug, K: Banarasi 5ug, L: Banarasi 10ug, M: Banarasi 25ug, N: Banarasi 50ug, O: Banarasi 100ug, P: Poona 5ug, Q: Poona 10ug, R: Poona 25ug**

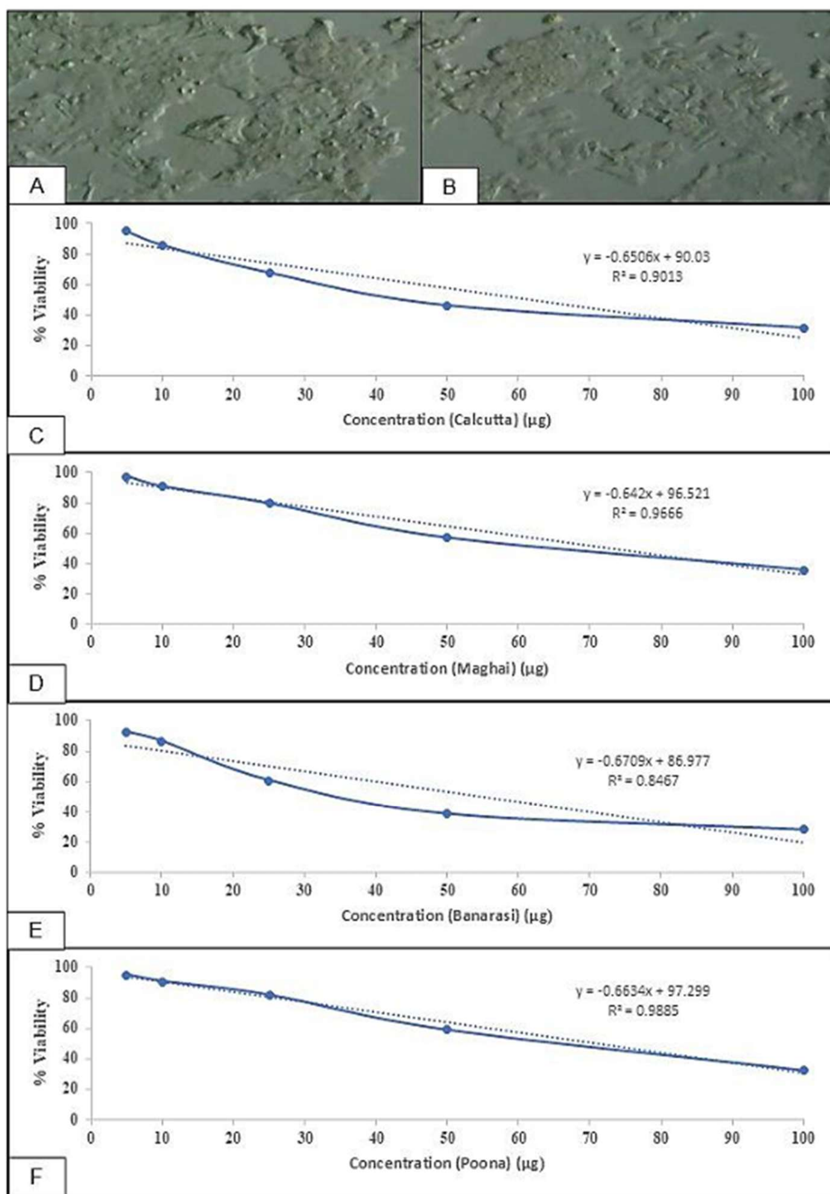


**Fig 4: Cytotoxic effect on increasing concentration of P. betle leaf extract of KB Cell lines; A: Poona 50ug, B: Poona 100ug, C: Percentage Viability of Calcutta, D: Percentage Viability of Maghai, E: Percentage Viability of Banarasi, F: Percentage Viability of Poona.**

**Table 1: Percentage of viability and $IC_{50}$ of cells with varying concentration of ethanolic extracts of *P. betle* (Calcutta, Maghai, Banarasi and Poona)**

| *P. Betle* leaves | Concentration (µg) | Concentration (µg) | Concentration (µg) | Concentration (µg) | Concentration (µg) |
|---|---|---|---|---|---|
| Calcutta | 5 | 0.493 | 0.507 | 0.508 | 0.502 |
| | 10 | 0.451 | 0.456 | 0.46 | 0.454 |
| | 25 | 0.359 | 0.361 | 0.354 | 0.358 |
| | 50 | 0.241 | 0.247 | 0.244 | 0.244 |
| | 100 | 0.172 | 0.163 | 0.164 | 0.166 |
| | Untreated | 0.526 | 0.528 | 0.531 | 0.528 |
| Maghai | Concentration (µg) | Absorbance at 570nm | Absorbance at 570nm | Absorbance at 570nm | Average |
| | 5 | 0.514 | 0.518 | 0.512 | 0.514 |
| | 10 | 0.479 | 0.475 | 0.483 | 0.479 |
| | 25 | 0.421 | 0.418 | 0.422 | 0.42 |
| | 50 | 0.297 | 0.302 | 0.306 | 0.301 |
| | 100 | 0.188 | 0.192 | 0.191 | 0.19 |
| | Untreated | 0.526 | 0.528 | 0.531 | 0.528 |
| Banarasi | Concentration (µg) | Absorbance at 570nm | Absorbance at 570nm | Absorbance at 570nm | Average |
| | 5 | 0.486 | 0.493 | 0.488 | 0.489 |
| | 10 | 0.453 | 0.458 | 0.462 | 0.457 |
| | 25 | 0.321 | 0.326 | 0.319 | 0.322 |
| | 50 | 0.203 | 0.208 | 0.205 | 0.205 |
| | 100 | 0.146 | 0.154 | 0.152 | 0.15 |
| | Untreated | 0.526 | 0.528 | 0.531 | 0.528 |
| Poona | Concentration (µg) | Absorbance at 570nm | Absorbance at 570nm | Absorbance at 570nm | Average |
| | 5 | 0.502 | 0.503 | 0.506 | 0.503 |
| | 10 | 0.486 | 0.479 | 0.477 | 0.48 |
| | 25 | 0.432 | 0.438 | 0.429 | 0.433 |
| | 50 | 0.311 | 0.315 | 0.314 | 0.313 |
| | 100 | 0.176 | 0.178 | 0.169 | 0.174 |
| | Untreated | 0.526 | 0.528 | 0.531 | 0.528 |

## V. SUMMARY

Oral cancer has become a worldwide threat. Many herbs are screened for anti-cancer potential and many of common household herbs like turmeric, ginger, saffron, cinnamon etc have shown efficacy Nazhvani et al. (2020). Piper betel has been previously explored for curing prostate cancer (Paranjpe, et al., 2013) owing to

presence of eugenol which is anti-carcinogenic compound (Singh, et al., 2023). Merchant and Pitiphat (2014) claimed that chewing of betel leaves with or without tobacco increased risk of oral cancer, on the other hand Veettil et al. (2022) claimed that home grown betel leaf was a potent anti-cancer agent.

## VI. CONCLUSION

During the present studies, $IC_{50}$ values were calculated for betel leaves. $IC_{50}$ value is the concentration of the extract of leaves required to inhibit 50% malignant cells. Comparing the four varieties of Betel leaves available in most regions of India, it was observed that Banarasi variety has the highest potential followed by Calcutta, Poona and Maghai respectively.

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## REFERENCES

1. Benzie, I. F., & Strain, S. (1996). The ferric reducing Ability of plasma (FRAP) as a measure of “Antioxidant power”: the FRAP assay. Analytical Biochemistry, 239(1), 70–76. https://doi.org/10.1006/abio.1996.0292
2. Bhuvaneswari, S., Sripriya, N., Deepa, S. and Prakash, N.U., 2014. Studies on antioxidant activities of six cultivars of *Piper betle* linn. Int. J. Pharm. Pharm. Sci, 6(11), pp.270-273.
3. Boontha, S., Taowkaen, J., Phakwan, T., Worauaicha, T., Kamonnate, P., Buranrat, B. and Pitaksuteepong, T., 2019. Evaluation of antioxidant and anticancer effects of *Piper betle* L (Piperaceae) leaf extract on MCF-7 cells, and preparation of transdermal patches of the extract. Tropical Journal of Pharmaceutical Research, 18(6), pp.1265-1272. http://dx.doi.org/10.4314/tjpr.v18i6.17
4. Dehghani Nazhvani, A., Sarafraz, N., Askari, F., Heidari, F., & Razmkhah, M. (2020). Anti-Cancer Effects of Traditional Medicinal Herbs on Oral Squamous Cell Carcinoma. Asian Pacific journal of cancer prevention: APJCP, 21(2), 479–48. https://doi.org/10.31557/APJCP.2020.21.2.479

5. Dinesh M.D., Athira P.S., Ajma N., Abhisha N.C. and Ashitha C., 2016. Anti-Cancer Activity (Oral) of Betel Leaf Extract by in-Vitro. International Journal of Life-Sciences Scientific Research. https://doi.org/10.4103/jomfp.jomfp_146_25
6. Fang, Y., Yu, X., & Jing, Y. (2017). Optimized preparation, characterization, and antioxidant activity of chitooligosaccharide–glycine Maillard reaction products. Journal of Food Science and Technology, 55(2), 712–720. https://doi.org/10.1007/s13197-017-2982-0
7. Guha, P., 2006. Betel leaf: the neglected green gold of India. Journal of Human Ecology, 19(2), pp.87-93. https://doi.org/10.22271/phyto.2024.v13.i2c.14886
8. Harini, S.S., Sougandhi, P.R., Tenkayala, D.S.R. and Gopinath, K.R., 2018. Antioxidant activity (phenol and flavonoid content) of three different cultivars of *Piper betle* l.(piperaceae). Journal of Drug Delivery and Therapeutics, 8(5-s), pp.143-148. https://doi.org/10.22270/jddt.v8i5-s.1978
9. Jaiswal, S.G., Patel, M., Saxena, D.K. and Naik, S.N., 2014. Antioxidant properties of *Piper betel* (L) leaf extracts from six different geographical domain of India. J. Bioresour. Eng. Technol, 1, pp.18-26.
10. Manigauha, A., Ali, H. and Maheshwari, M.U., 2009. Antioxidant activity of ethanolic extract of *Piper betel* leaves. J Pharm Res, 2(3), pp.194-95.
11. Merchant, A. T., & Pitiphat, W. (2014). Total, direct, and indirect effects of paan on oral cancer. Cancer Causes & Control, 26(3), 487–491. https://doi.org/10.1007/s10552-014-0516-x
12. Mukherjee, P. K. (2019). Quality control and evaluation of herbal drugs: Evaluating Natural Products and Traditional Medicine. Elsevier.
13. Paranjpe, R., Gundala, S. R., Lakshminarayana, N., Sagwal, A., Asif, G., Pandey, A., & Aneja, R. (2013). *Piper betle* leaf extract: anticancer benefits and bio-guided fractionation to identify active principles for prostate cancer management. Carcinogenesis, 34(7), 1558–1566. https://doi.org/10.1093/carcin/bgt066
14. Pradhan, D., Suri, K.A., Pradhan, D.K. and Biswasroy, P., 2013. Golden heart of the nature: *Piper betle* L. Journal of Pharmacognosy and Phytochemistry, 1(6), pp.147-167.

15. Rintu, D., Shinjini, M., Kaustab, M., Pramathadhip, P., Umesh, P.S. and Banerjee, E.R., 2015. Anti oxidant and anti-inflammatory activities of different varieties of *Piper* leaf extracts (*Piper betle* L.). J Nutr Food Sci, 5(415), pp.2. https://doi.org/10.4172/2155-9600.1000415
16. Sankeshwari, R., Ankola, A. V., Bhat, K., & Hullatti, K. (2018). Soxhlet versus cold maceration: Which method gives better antimicrobial activity to licorice extract against Streptococcus mutans. Journal of the Scientific Society, 45(2), pp. 67. https://doi.org/10.4103/jss.JSS_27_18
17. Singh, T., Singh, P., Pandey, V. K., Singh, R., & Dar, A. H. (2023). A literature review on bioactive properties of betel leaf (*Piper betle* L.) and its applications in food industry. Food Chemistry Advances, 3, 100536. https://doi.org/10.1016/j.focha.2023.100536
18. Smila, K.H., Priyanka, J.D., Srinithya, B., Sripriya, N. and Udaya Prakash, N.K., 2014. Studies on phytochemistry and bioefficacy of cultivars of *Piper betle* Linn. Int J Res Pharm Sci, 5(2), pp.1-5.
19. Uddin, M.F., Uddin, S.A., Hossain, M.D. and Manchur, M.A., 2015. Antioxidant, cytotoxic and phytochemical properties of the ethanol extract of *Piper betle* leaf. https://doi.org/10.13040/IJPSR.0975 8232.6
20. Veettil, S. R., Sunil, E. A., Mukunda, A., Mohan, A., John, S., & Pynadath, M. K. (2022). Anticancer effect of *Piper betle* leaf extract on KB cell lines – an in vitro study. Oral and Maxillofacial Pathology Journal, 28–31. https://www.ompj.org/archives
21. Venkanna, A., Siva, B., Poornima, B., Vadaparthi, P.R., Prasad, K.R., Reddy, K.A., Reddy, G.B.P. and Babu, K.S., 2014. Phytochemical investigation of sesquiterpenes from the fruits of *Schisandra chinensis* and their cytotoxic activity. Fitoterapia, 95, pp.102-108. https://doi.org/10.1016/j.fitote.2014.03.003
22. Venugopalan, S., Sharma, A., Venugopalan, V. and Gautam, H.K., 2015. Comparative study on the antioxidant activities of extracts from *Piper betle* leaves. Biomedical and Pharmacology Journal, 1(1), pp.115-120.