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Evaluation of antioxidant property and anticancer prospective of the leaf extract and biofractions of *Bauhinia foveolata* Dalzell – A native of the Indian Western Ghats

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ABSTRACT

The current study was designed to evaluate the antioxidant and anticancer potential of ethanolic leaf extract of *Bauhinia foveolata* Dalzell. (EEBF) and its toluene, ethyl acetate and methanolic biofractions viz., TFBF, EFBF and MFBF. Phytoconstituents were screened by adopting established procedures. Total phenolic and flavonoid content were assessed spectrophotometrically. *In vitro* antioxidant activity was assayed using DPPH (2,2-diphenyl-1-picrylhydrazyl), hydrogen peroxide and nitric oxide as free radicals, whereas anticancer activity was evaluated using sulforhodamine B assay. EEBF showed maximum phenolic content of 49.12±0.31 mg GAE/g and flavonoidal content of 28.75±0.42 mg QUE/g, than its biofractions. EEBF showed considerable antioxidant activity with IC_{50}=19.04±0.24 μg/mL and IC_{50}=65.85±1.22 μg/mL when compared to the standards Ascorbic acid (IC_{50}=12.06±0.05 μg/mL) and Gallic acid (IC_{50}=64.65±0.72 μg/mL) in DPPH and nitric oxide scavenging assays, respectively. MFBF showed significant activity with IC_{50}=26.76±0.75 μg/mL in hydrogen peroxide scavenging assay compared to the standard Gallic acid (IC_{50}=76.60±1.31 μg/mL). TFBF showed favourable growth inhibition of MCF-7 cells with GI_{50}=73.5±11.96 μg/mL when compared to other samples screened (GI_{50}>80 μg/mL) as against the standard Adriamycin (GI_{50}<10 μg/mL) in SRB assay. The therapeutic virtues of EEBF and MFBF as free radical scavengers and TFBF as an antiproliferative may be attributed to the phenolics, flavonoids, steroids and triterpenoids present.

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INTRODUCTION

Reactive free radicals contribute to oxidative stress in the living cells by damaging the lipids, proteins, DNA and other macromolecular components (Pham-Huy *et al.*, 2008). Altered cellular changes and excitotoxicity play a specific feature in the pathogenesis of degenerative diseases like cancer, cardiovascular diseases, neurodegenerative disorders, cataract and inflammation (Nely *et al.*, 2020). Cancer is a life-threatening disease that can potentially diminish the quality of life of an individual. Breast cancer is a growing concern nowa-

days, as it is a leading cause of cancer-related mortalities amongst women worldwide (Malvia *et al.*, 2017; Mathur *et al.*, 2020). It can be clinically sub-classified based on the underlying molecular mechanisms viz., gene expression and receptor status (estrogen receptor [ER], progesterone receptor [PR], human epidermal growth factor receptor 2 [HER2]) and proliferation status (Medeiros and Allan, 2019). Constant research is being carried out to identify agents that may help alleviate the symptoms and treat breast cancer.

Consumption of antioxidant-rich food helps to minimize the chances of the development of degenerative diseases (Do *et al.*, 2014). Potential free radical scavengers may thus help reduce cell death.

Plants rich in essential phytoconstituents are being extensively investigated for their medicinal potential to treat various ailments. Several species of the genus *Bauhinia* are widely distributed across Africa, Asia and South America and have been used frequently in folk medicine to treat different kinds of pathologies (Filho, 2009). *Bauhinia foveolata* Dalzell. (family Leguminosae) is usually found along the Western Ghats of India. The tree is dioecious and grows to a height of about 30 meters. Leaves are suborbicular to broadly ovate and pubescent beneath numerous closely situated fine pits within areolae of reticulations (Bandyopadhyay *et al.*, 2005). The synonyms of *B. foveolata* are *Bauhinia lawii* Baker and *Piliostigma foveolatum* (Dalzell) Thoth, and its common name is Pore leaved *Bauhinia.* Its leaves are possibly the largest among all *Bauhinia* species found in India, and its scientific name *foveolata* is due to the presence of minute pores (foveoli) on the underside of the leaves (Bandyopadhyay *et al.*, 2005; Habbu *et al.*, 2020). Very few studies have been documented on *B. foveolata* Dalzell. A recent study reported the isolation of quercetin and odoratin-7-glucoside from butanol fraction of ethanolic leaf extract of *B. foveolata* that exhibited cytotoxicity towards the human colon cancer cell lines, HT-29 and HCT-15 (Habbu *et al.*, 2020). The acetone and ethyl acetate extracts of *B. foveolata* bark reported antibacterial and antimalarial activities, respectively (Gamit *et al.*, 2018). The commonly found species of *Bauhinia* in India have reported antioxidant, antimicrobial and cytotoxic effects (Mishra *et al.*, 2013; Vijayan *et al.*, 2019).

Thus, the current study was focused on evaluating the antioxidant and anticancer potential of the ethanolic leaf extract of *B. foveolata* Dalzell. (EEBF) and its toluene, ethyl acetate and methanol soluble fractions, i.e., TFBF, EFBF and MFBF by *in vitro* assays.

MATERIALS AND METHODS

Chemicals and reagents

High purity analytical grade solvents and chemicals were used for the study. Folin ciocalteau reagent, aluminum chloride, DPPH, sodium nitroprusside, ferrous ammonium sulfate, and sulphanilamide were purchased from Molychem (Mumbai, India). 1,10-phenanthroline and hydrogen peroxide solution were purchased from Merck (Bangalore, India). Naphthyethylene diamine was procured from Qualigens (Mumbai, India). Reference standards were procured from Sigma-Aldrich (St. Louis, Mo., USA).

Instrumentation

A double beam UV-Vis spectrophotometer (LABINDIA Analytical UV 3092) was used for spectroscopic analysis.

Plant material

Fresh leaves of *B. foveolata* were collected from the forests of Dandeli, Karnataka – India, in the months of August-September 2017. They were washed to remove the extraneous matter and then dried. Dr. Satyanarayan S. Hebbar, Department of Botany, Government Pre university college, Dharwad, Karnataka, authenticated the specimen (GCPDOP-07) which was then deposited in the Department.

Preparation of experimental samples

The experimental samples consisted of the ethanolic leaf extract of *B. foveolata* (EEBF) and its three biofractions viz., toluene soluble fraction of EEBF (TFBF), ethyl acetate soluble fraction of EEBF (EFBF) and methanol soluble fraction of EEBF (MFBF).

Preparation of the ethanolic leaf extract of B. foveolata (EEBF)

1.5 kg of powdered leaves were defatted by refluxing with 3.5 L of Petroleum ether thrice. The defatted drug (900g) was refluxed with absolute ethanol (4.5L) for 90 minutes, thrice. The alcoholic extract was filtered using the Whatmann filter paper (No.1). The solvent from the extract was distilled off using a rotary evaporator and air-dried to yield the dry extract (79.14g). The ethanolic extract of *B. foveolata* (EEBF) was stored at 4°C until further processed.

Preparation of biofractions of EEBF

20 g of EEBF was refluxed successively with toluene, ethyl acetate and methanol and filtered using Whatmann filter paper (No.1). The solvent was distilled off using a rotary evaporator and the toluene, ethyl acetate and methanol soluble fractions of the

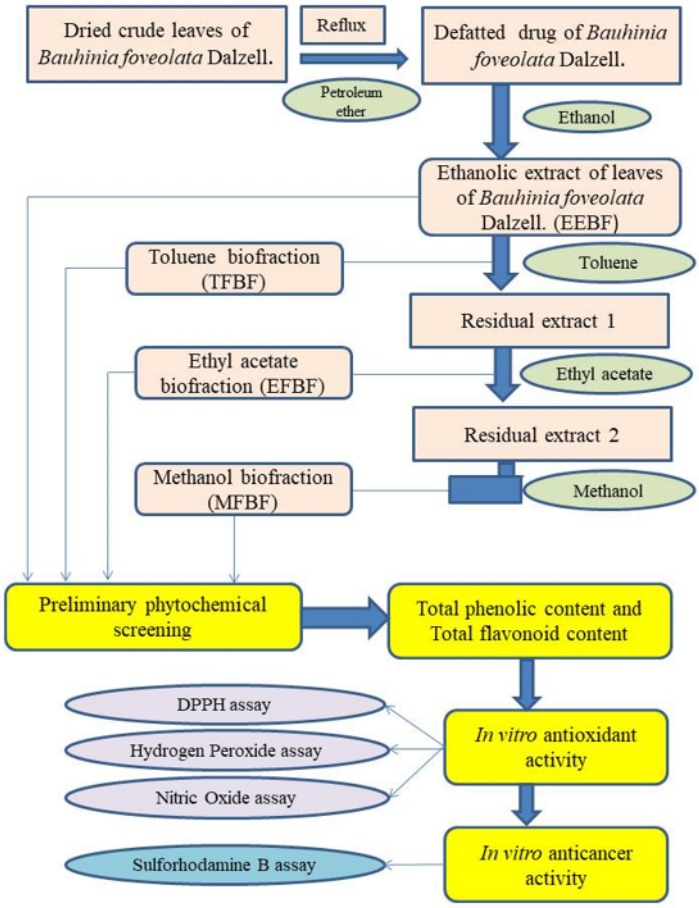

Figure 1: Schematic representation of the process.

ethanolic leaf extract of *B. foveolata* viz., TFBF (5.14g), EFBF (3.36g) and MFBF (6.91g) were successively obtained and stored at 4°C unil further used (Figure 1).

Qualitative phytochemical screening

The experimental samples (EEBF, TFBF, EFBF and MFBF) were screened to detect the phytoconstituents present by adopting standard procedures (Khandelwal, 2010).

Quantitative phytochemical analysis

The total phenolic content, total flavonoid content, *in vitro* antioxidant and anticancer activities were carried out with slight modifications to the referenced procedures.

Total Phenolic Content

The total phenolic content of the experimental samples was determined using the Folin-Ciocalteau's method (De Oliveira *et al.*, 2012). The intensity of blue colour formed due to the polyphenol content in different concentrations (10 - 1000 μg/mL) of experimental samples was measured at 760 nm using UV-Visible spectrophotometer. Various concentrations (10-100 μg/mL) of gallic acid were used to plot the standard curve. The total phenolic content of EEBF and its biofractions were expressed as mg of GAE/g of samples.

Total Flavonoid content

The total flavonoid content of the experimental samples was estimated using the aluminum chloride colorimetry method (Meda *et al.*, 2005). The reactions of varying concentrations (10 - 1000 μg/mL) of experimental samples were analyzed at an absorbance of 415nm using a UV-VIS spectrophotometer. The standard graph was plotted with a series of concentrations (10-100 μg/mL) of quercetin on the X-axis and absorbance on the Y-axis. The total flavonoid content of EEBF and its biofractions was determined from the standard graph and expressed as mg of QUE equivalents/g of samples.

In vitro antioxidant activity

The *in vitro* antioxidant activity of the selected experimental samples (EEBF, TFBF, EFBF and MFBF) was measured using the DPPH, hydrogen peroxide and nitric oxide radical scavenging methods.

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay

The radical scavenging assay of the selected samples was studied using DPPH free radicals (Chanda and Dave, 2009).

The absorbances of the reaction mixtures that consisted of 1 mL of DPPH in methanol (0.3 mM), 1 mL of varying concentrations of experimental samples (10 - 1000 μg/mL) or standard drug Ascorbic acid (2 - 20 μg/mL) and 1 mL of methanol, was measured at 517 nm using a UV- Visible spectrophotometer, after incubation for 30 min in the dark, at room temperature.

The percentage of DPPH scavenged was calculated by using the formula,

$$\% \; DPPH \; scavenged = \left[\frac{Absorbance \; of \; control - \; Absorbance \; of \; sample}{Absorbance \; of \; control}\right] \times 100$$

Hydrogen peroxide radical scavenging assay

In this assay, the reaction mixture consisted of 0.3mL of ferrous ammonium sulfate (1 mM), different concentrations of experimental samples (10 - 100 μg/mL) or standard drug Gallic acid (10 - 100 μg/mL), 0.1 mL of hydrogen peroxide (5 mM) and 1.5 mL of 1,10-phenanthroline (1mM) (Mukhopadhyay *et al.*, 2016).

After appropriate incubation, the absorbance of the reaction mixture was analysed at a wavelength of 510 nm using a UV- Visible spectrophotometer.

The percentage of hydrogen peroxide free radical scavenged was calculated by using the formula

$$\% \; H_2O_2 \; scavenged = \left[\frac{Absorbance \; of \; sample}{Absorbance \; of \; control}\right] \times 100$$

Nitric oxide radical scavenging (NO) assay

The amount of nitric oxide free radicalsscavenged by the treatment groups was determined (Boora *et al.*, 2014).

The absorbance of the reaction mixtures that consisted of 1mL of sodium nitroprusside solution with 1 mL of different concentrations of experimental samples (10 - 1000 μg/mL) or standard drug Gallic acid (10 - 100 μg/mL), incubated in the dark at 25°C for 180 min, successively treated with 2mL of freshly prepared Griess reagent and further incubated for 20 min was measured using a UV- Visible spectrophotometer at a wavelength of 540 nm.

The percentage of free radical nitric oxide scavenged was calculated by using the formula,

$$\% \; Nitric \; Oxide \; scavenged = \left[\frac{Absorbance \; of \; control - \; Absorbance \; of \; sample}{Absorbance \; of \; control}\right] \times 100$$

In vitro anticancer activity

The *in vitro* anticancer activity of the experimental samples was determined using SRB assay (Lopez *et al.*, 2018; Skehan *et al.*, 1990; Vanicha Vichai and Kirtikara, 2006). RPMI 1640 media containing 10% fetal bovine serum along with 2mM L-glutamine were used to grow the MCF-7 cell lines, which were further inoculated into 96 well microtiter plates in 100 μL at plating densities of 1x10^{3}cells/well and incubated at 37°C, 5% CO_2, 95% air and 100% relative humidity for 24 h before addition of experimental samples (EEBF and its biofractions) or standard drug Adriamycin. Standard drug and experimental samples were solubilized in dimethyl sulfoxide at 100mg/mL and diluted with water. Varying concentrations of 10 μg/mL, 20 μg/mL, 40 μg/mL and 80 μg/mL of each experimental sample or standard drug were added to the microtiter wells and incubated at 37°C for 48 h. The assay was concluded by addition of cold trichloroacetic acid. About 50μL of trichloroacetic acid (30%w/v) was added to fix the cells in situ and incubated at 4°C for 60 min. After incubation, cells were washed, air-dried and stained with sulforhodamine B (SRB) for 20 mins and then washed with 1% acetic acid to remove excess stain. Tris buffer (10 mmol/L) was used to dissolve the bound sulforhodamine B and absorbance was measured on a plate reader at a wavelength of 540 nm. The average absorbance of test and control well was calculated and the percentage growth of MCF-7 cells was expressed using the formula,

$$\% \; growth = \left[\frac{Absorbance \; of \; test \; well}{Absorbance \; of \; control \; wells}\right] \times 100$$

Statistical analysis

Analyses were performed in triplicate observations and recorded. Data was documented as mean value $\pm$ standard deviation (SD). Calculation of IC_{50} and GI_{50} values were done using GraphPad Prism version 7.3. Statistical analysis was done using IBM© SPSS version 23.0 statistical software (IBM Corporation, New York, USA). One way Analysis of Variance (ANOVA) was used to measure statistical significance along with Duncan's multiple test (P <0.05 for antioxidant activity and <0.001 for anticancer activity).

RESULTS

Preliminary phytochemical analysis

The ethanolic leaf extract of *B. foveolata* (EEBF) and its experimental biofractions have confirmed the presence of alkaloids, flavonoids, tannins and phenolic compounds. Steroids and triterpenoids

Table 1: Preliminary phytochemical analysis of ethanolic leaf extract of *B. foveolata* and its biofractions.

Sr. No.	Phytochemical constituents	EEBF	TFBF	EFBF	MFBF
1.	Alkaloids	+ve	+ve	+ve	+ve
2.	Glycosides	+ve	-ve	-ve	+ve
3.	Saponins	+ve	-ve	-ve	+ve
4.	Carbohydrates	+ve	-ve	-ve	+ve
5.	Flavanoids	+ve	+ve	+ve	+ve
6.	Proteins	+ve	-ve	-ve	+ve
7.	Tannins and Phenolics	+ve	+ve	+ve	+ve
8.	Resins	-ve	-ve	-ve	-ve
9.	Steroids And Triterpenoids	+ve	+ve	-ve	-ve
10.	Starch	-ve	-ve	-ve	-ve

Where -ve indicates absent, +ve indicates present.

Table 2: Total phenolic content by Folin-ciocalteu method and Total flavonoid content by aluminum chloride method.

Method	EEBF	TFBF	EFBF	MFBF
Total phenolic content (mg GAE/g)	49.12±0.31	3.27±0.35	21.01±0.35	25.65±0.21
Total flavonoid content (mg QUE/g)	28.75±0.42	1.14±0.28	11.12±0.46	14.83±0.31

Each value is expressed as mean ± SD (n=3).

Table 3: *In vitro* antioxidant activity - DPPH, hydrogen peroxide and nitric oxide free radical scavenging activity.

Free radical scavenging assay	IC_{50} values (μg/mL) Standard	EEBF	TFBF	EFBF	MFBF
DPPH	12.06 ± 0.05^{a}	19.04 ± 0.24^{b}	391.5 ± 3.32^{e}	68.39 ± 0.27^{d}	53.44 ± 0.83^{c}
H_2O_2	76.60 ± 1.31^{a}	43.96 ± 0.92^{d}	71.45 ± 1.03^{b}	64.66 ± 0.43^{c}	26.76 ± 0.75^{e}
NO	64.65 ± 0.72^{a}	65.85 ± 1.22^{a}	569.67 ± 4.19^{c}	288.67 ± 10.61^{b}	71.63 ± 0.19^{a}

Each value is articulated as the mean ± SD (n= 3). Within each row, the means with different superscript letters are statistically significant (ANOVA, $P<0.05$, and subsequent post hoc multiple comparisons with Duncan's test).

Table 4: *In vitro* anticancer activity on human breast cancer cell line MCF-7

Experimental sample/ standard	% Control Growth of MCF-7 cell lines 10 μg/mL	20 μg/mL	40 μg/mL	80 μg/mL
EEBF	92.2 ± 3.59^{b}	$91.6\pm6.16b^{c}$	85.5 ± 3.77^{c}	74.7 ± 11.45^{b}
TFBF	88.9 ± 9.13^{b}	82.1 ± 6.50^{b}	67.8 ± 5.57^{b}	47.0 ± 10.67^{b}
EFBF	93.5 ± 5.70^{b}	93.3 ± 10.5^{bc}	90.7 ± 10.2^{c}	67.1 ± 9.4^{b}
MFBF	104.7 ± 3.89^{b}	111.1 ± 8.72^{c}	114.9 ± 4.72^{d}	115.4 ± 8.09^{c}
Standard (Adriamycin)	-61.2 ± 5.26^{a}	-64.9 ± 2.34^{a}	-50.5 ± 6.98^{a}	-18.8 ± 21.17^{a}

Each value is expressed as the mean ± SD (n =3). Within each column, the means with different superscript letters are statistically significant (ANOVA, $P<0.001$,and subsequent post hoc multiple comparisons with Duncan's test).

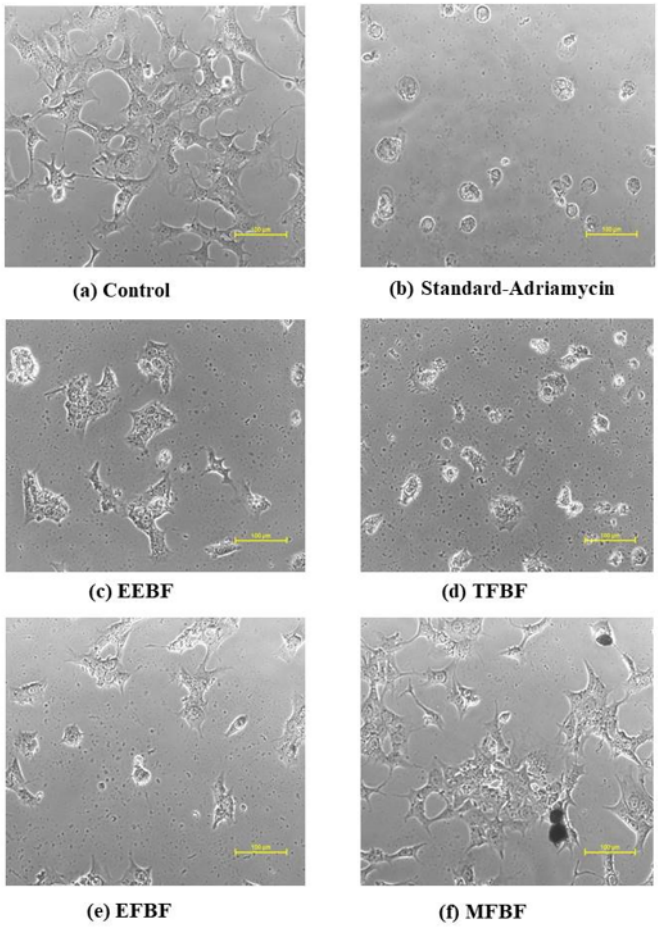

Figure 2: Morphology of (a) control and (b-f) treatment groups of standand and experimental samples at a concentration of 80μg/mLagainst MCF7 breast cancer cells.

were present in EEBF and its toluene biofraction (Table 1).

Estimation of the total phenolic and total flavonoid content

The ethanolic leaf extract of *B. foveolata* (EEBF) has reported the highest phenolic and flavonoid content, followed by its methanolic biofraction, as depicted in Table 2.

Determination of *in vitro* free radical scavenging activity

The results of the *in vitro* free radical scavenging activities performed using DPPH, hydrogen peroxide and nitric oxide free radicals have been listed in Table 3. IC_{50} value indicates the concentration of the sample, at which 50% of the free radicals are scavenged.

Determination of *in vitro* anticancer activity

The percent growth control of MCF-7 breast cancer cells observed with the selected experimental samples and standard drug Adriamycin by sulforhodamine B (SRB) assay, have been noted in Table 4. The morphological characteristics of MCF-7 breast cancer cells when treated with 80μg/mL of standard drug Adriamycin and each experimental sample have been illustrated in Figure 2.

DISCUSSION

Antioxidants are beneficial in reducing oxidative damage to cells and improving an individual's quality of life by preventing or postponing the onset of degenerative diseases like cancer, cardiovascular disease, neural disorders, Alzheimer's disease, mild cognitive impairment and Parkinson's disease (Alam *et al.*, 2013). Oxidative damage to cells may be attributed to the presence of free radicals and antioxidants limit this damage by donating electrons to free radicals and neutralizing them (Lalhminghlui and Jagetia, 2018). Endogenous and exogenous antioxidants play an important role in preventing oxidative stress (Bouayed and Bohn, 2010). Plants, vegetables and fruits have been known to be a source of exogenous antioxidants that include phenolics, carotenoids, anthocyanins and tocopherols (Altemimi *et al.*, 2017; Kasote *et al.*, 2015).

The preliminary phytochemical investigations of the experimental samples of ethanolic leaf extracts of *B. foveolata* Dalzell. (EEBF) and its bio fractions viz. TFBF, EFBF and MFBF revealed the presence of various phytoconstituents. Naturally occurring polyphenolics have gained popularity due to the high antioxidant, anti-inflammatory and anti-carcinogenic potential that help reduce the risk of life-threatening diseases (Zakaria *et al.*, 2011). Flavonoids, tannins and phenolic compounds exhibit antioxidant activity mainly due to their redox properties, since they behave as reducing agents, hydrogen donating agents or singlet oxygen quenching agents (Medini *et al.*, 2014; Patel *et al.*, 2011). Folin ciocalteau method and aluminum chloride method were used to quantitatively estimate the phenolic and flavonoid contents, respectively. EEBF showed the maximum phenolic content and flavonoid content as compared to its biofractions. *In vitro* antioxidant activity was assessed using three radicals viz. DPPH (2,2-diphenyl-1-picrylhydrazyl), hydrogen peroxide and nitric oxide. A lower IC_{50} value indicates higher scavenging ability of the extract and hence better antioxidant activity (Brighente *et al.*, 2007). DPPH, hydrogen peroxide and nitric oxide free radical scavenging assays were selected as the models to study the *in vitro* antioxidant activity of the ethanolic leaf extract and biofractions of B. *foveolata* Dalzell., as they are ideal methods to screen natural products (Alam *et al.*, 2013; Parul *et al.*, 2013). EEBF has exhibited significant ($p<0.05$) antioxidant potential in DPPH and nitric oxide radical scavenging methods. Interestingly, the methanolic biofraction (MFBF) displayed significant *in vitro* antioxidant potential in hydrogen peroxide free radical scavenging assay. The antioxidant activity of EEBF and MFBF may thus be attributed to the presence of phenolics and flavonoids.

The anticancer properties of natural foods are possibly due to the additive and synergistic effects of various phytochemicals (Mates *et al.*, 2011). Steroids have the potential to act as cytostatic (antiproliferative) and cytotoxic anticancer agents. Steroidal hormones like estrogen, progesterone and androgen play a crucial role in developing secondary sexual characteristics in females and males. Breast cancer and other steroidal hormone related carcinogenesis mainly occur due to accelerated cell proliferation. Steroidal anticancer drugs act as antihormonal antiproliferative through enzyme inhibition (steroid sulfatase inhibitors, aromatase inhibitors (AI), hydroxysteroid dehydrogenase inhibitors) or receptor inhibition (antiestrogens, antiprogestins). Their cytotoxic action is mainly exhibited by naturally occurring or semisynthetic compounds through non-hormonal targets (Gupta *et al.*, 2013). Natural triterpenes have proved to possess anti-oxidative, anticancer and chemopreventive effects and show cytotoxicity against tumor cells with low activity towards normal cells (Chudzik *et al.*, 2015).

In vitro anticancer activity was assessed by sulforhodamine B (SRB) assay, using MCF-7 breast cancer cell lines. Amongst the experimental samples subjected to *in vitro* anticancer activity, TFBF showed satisfactory growth inhibition of MCF-7 cells at GI_{50}=73.5+11.96 μg/mL. TFBF was found to be substantively rich in steroids and triterpenoids. Thus, the antiproliferative property of TFBF could be attributed to the presence of steroids, triterpenoids that may also have synergetic effects with other phytoconstituents present.

CONCLUSIONS

The study effectively concludes that the ethanolic leaf extract of *B. foveolata* Dalzell. (EEBF) and its methanolic biofraction (MFBF) have encouraging antioxidant activity as EEBF substantially scavenged DPPH and nitric oxide, while MFBF considerably neutralized hydrogen peroxide free radicals *in vitro.* Notably, the toluene biofraction effectively inhibited the growth of MCF-7 human breast cancer cells in sulforhodamine B assay, thus demonstrating its antiproliferative potential against breast cancer. However, further phytochemical and pharmacological investigation of EEBF and its biofractions are desirable to determine the active compounds and deduce their mechanisms of action.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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REFERENCES

Alam, M. N., Bristi, N. J., Rafiquzzaman, M. 2013. Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal*, 21(2):143–152.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D., Lightfoot, D. 2017. Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. *Plants*, 6(4):42.

Bandyopadhyay, S., Thothathri, K., Sharma, B. D. 2005. The genus Bauhinia L. (Leguminosae: Caesalpinioideae) in India. *Journal of Economic and Taxonomic Botany*, 29(4):763–801.

Boora, F., Chirisa, E., Mukanganyama, S. 2014. Evaluation of Nitrite Radical Scavenging Properties of Selected Zimbabwean Plant Extracts and Their Phytoconstituents. *Journal of Food Processing*, 2014:1–7.

Bouayed, J., Bohn, T. 2010. Exogenous Antioxidants—Double-Edged Swords in Cellular Redox State: Health Beneficial Effects at Physiologic Doses versus Deleterious Effects at High Doses. *Oxidative Medicine and Cellular Longevity*, 3(4):228–237.

Brighente, I. M. C., Dias, M., Verdi, L. G., Pizzolatti, M. G. 2007. Antioxidant Activity and Total Phenolic Content of Some Brazilian Species. *Pharmaceutical Biology*, 45(2):156–161.

Chanda, S., Dave, R. 2009. In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. *African Journal of Microbiology Research*, 3(13):981–996.

Chudzik, M., Korzonek-Szlacheta, I., Król, W. 2015. Triterpenes as Potentially Cytotoxic Compounds. *Molecules*, 20:1610–1625.

De Oliveira, A. M. F., Pinheiro, L. S., Pereira, C. K. S., Matias, W., Gomes, R., Chaves, O. S., Vanderlei De Souza, M. D. F., De Almeida, R. N. 2012. Simões de Assis, T. 2012. Total phenolic content and antioxidant activity of some Malvaceae family species. *Antioxidants*, 1(1):33–43.

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., Ju, Y.-H. 2014. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. *Journal of Food and Drug Analysis*, 22(3):296–302.

Filho, V. C. 2009. Chemical composition and biological potential of plants from the genus Bauhinia. *Phytotherapy Research*, 23(10):1347–1354.

Gamit, S. B., Sapra, P., Vasava, M. S., Solanki, H. A., Patel, H., Rajani, D. 2018. Antimicrobial and antimalarial activities of some selected ethnomedicinal plants used by tribal communities of Tapi District, Gujarat, India. *International Research Journal of Pharmacy*, 9(10):151–156.

Gupta, A., Kumar, B. S., Negi, A. S. 2013. Current status on development of steroids as anticancer agents. *The Journal of Steroid Biochemistry and Molecular Biology*, 137:242–270.

Habbu, P. V., Miskin, N., Kulkarni, V. H., Bhat, P., Joshi, A., Bhandarkar, A., Kulkarni, S., Dixit, S. R., Vaijanathappa, J. 2020. Isolation, characterization, and cytotoxic studies of secondary metabolites from the leaves of Bauhinia foveolata Dalzell: An endemic tree from the Western Ghats, India. *Journal of Applied Pharmaceutical Science*, 10(03):135–148.

Kasote, D. M., Katyare, S. S., Hegde, M. V., Bae, H. 2015. Significance of Antioxidant Potential of Plants and its Relevance to Therapeutic Applications. *International Journal of Biological Sciences*, 11(8):982–991.

Khandelwal, K. R. 2010. Practical Pharmacognosy. *Techniques and Experiments*. 20th Edn. Nirali Prakashan.

Lalhminghlui, K., Jagetia, G. C. 2018. Evaluation of the free-radical scavenging and antioxidant activities of Chilauni, Schima wallichii Korth in vitro. *Future Science OA*, 4(2):FSO272.

Lopez, D., Cherigo, L., de Sedas, A., Spadafora, C., Martinez-Luis, S. 2018. Evaluation of antiparasitic, anticancer, antimicrobial and hypoglycemic properties of organic extracts from Panamanian mangrove plants. *Asian Pacific Journal of Tropical Medicine*, 11(1):32.

Malvia, S., Bagadi, S. A., Dubey, U. S., Saxena, S. 2017. Epidemiology of breast cancer in Indian women. *Asia-Pacific Journal of Clinical Oncology*, 13(4):289–295.

Mates, J. M., Segura, J. A., Alonso, F. J., Marquez, J. 2011. Anticancer Antioxidant Regulatory Functions of Phytochemicals. *Current Medicinal Chemistry*, 18(15):2315–2338.

Mathur, P., Sathishkumar, K., Chaturvedi, M., Das, P., Sudarshan, K. L., Santhappan, S., Nallasamy, V., John, A., Narasimhan, S., Roselind, F. S. 2020. Cancer Statistics, 2020: Report from National Cancer Registry Programme, India. *JCO Global Oncology*, 6(6):1063–1075.

Meda, A., Lamien, C. E., Romito, M., Millogo, J., Nacoulma, O. G. 2005. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scaveng-

ing activity. *Food Chemistry*, 91(3):571–577.

Medeiros, B., Allan, A. L. 2019. Molecular Mechanisms of Breast Cancer Metastasis to the Lung: Clinical and Experimental Perspectives. *International Journal of Molecular Sciences*, 20(9):2272.

Medini, F., Fellah, H., Ksouri, R., Abdelly, C. 2014. Total phenolic, flavonoid and tannin contents and antioxidant and antimicrobial activities of organic extracts of shoots of the plant Limonium delicatulum. *Journal of Taibah University for Science*, 8(3):216–224.

Mishra, A., Sharma, A. K., Kumar, S., Saxena, A. K., Pandey, A. K. 2013. Bauhinia variegataLeaf Extracts Exhibit Considerable Antibacterial, Antioxidant, and Anticancer Activities. *BioMed Research International*, 2013:1–10.

Mukhopadhyay, D., Dasgupta, P., Roy, D. S., Palchoudhuri, S., Chatterjee, I., Ali, S., Dastidar, S. G. 2016. A Sensitive In vitro Spectrophotometric Hydrogen Peroxide Scavenging Assay using 1,10-Phenanthroline. *Free Radicals and Antioxidants*, 6:124–132.

Nely, M. M., Jenny, P. C., Yanet, C. O., Ruben, D. S., Carla, L. D., Luis, A. F. 2020. Evaluation of antioxidant potential and total phenolic content of exotic fruits grown in Colombia. *Journal of Applied Pharmaceutical Science*, 10(09):50–58.

Parul, R., Kundu, S. K., Saha, P. 2013. In vitro nitric oxide scavenging activity of methanol extracts of three Bangladeshi medicinal plants. *The Pharma Innovation-Journal*, 1(12):83–88.

Patel, D. K., Kumar, R., Prasad, S. K., Hemalatha, S. 2011. Pedalium murex Linn (Pedaliaceae) fruits: a comparative antioxidant activity of its different fractions. *Asian Pacific Journal of Tropical Biomedicine*, 1(5):395–400.

Pham-Huy, L. A., He, H., Pham-Huy, C. 2008. Free radicals, antioxidants in disease and health. *International Journal of Biomedical Science*, 4(2):3614697.

Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J. T., Bokesch, H., Kenney, S., Boyd, M. R. 1990. New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening. *JNCI Journal of the National Cancer Institute*, 82(13):1107–1112.

Vanicha Vichai, Kirtikara, K. 2006. Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nature Protocols*, 1(3):1112–1116.

Vijayan, R., Joseph, S., Mathew, B. 2019. Anticancer, antimicrobial, antioxidant, and catalytic activities of green-synthesized silver and gold nanoparticles using Bauhinia purpurea leaf extract. *Bioprocess and Biosystems Engineering*, 42(2):305–319.

Zakaria, Z. A., Rofiee, M. S., Teh, L. K., Salleh, M. Z., Sulaiman, M. R., Somchit, M. N. 2011. Bauhinia purpurea leaves' extracts exhibited in vitro antiproliferative and antioxidant activities. *African Journal of Biotechnology*, 10(1):65–74.

