

## ***In vitro* Cytotoxic Potential of Alkaloid and Flavonoid Rich Fractions of *Alseodaphne semecarpifolia* Against MCF-7 Cells**

**Ganadhali Puttaramaiah Chethankumara<sup>1</sup>,  
Kakanahalli Nagaraj<sup>1\*</sup> and Venkatarangaiah Krishna<sup>2</sup>**

<sup>1</sup>Department of PG Studies and Research in Applied Zoology, Kuvempu University, Shivamogga, Karnataka - 577 451, India.

<sup>2</sup>Department of PG Studies and Research in Biotechnology, Kuvempu University, Shivamogga, Karnataka - 577 451, India.

\*Corresponding author E-mail: knagarajv@gmail.com

<https://dx.doi.org/10.13005/bpj/2158>

(Received: 25 January 2021; accepted: 17 April 2021)

The stem bark and leaves of *A. semecarpifolia* (Lauraceae) have been employed by traditional healers in Western Ghats region of Karnataka, India to treat human breast cancer. The present study was initiated to explore the cytotoxic properties of *A. semecarpifolia*. The secondary metabolites were extracted from stem bark and leaves. The stem bark methanol extract (SBME) and leaf methanol extract (LME) were subjected to liquid-liquid partition chromatography, followed by evaluating the presence of phytochemical constituents in liquid fractions and their cytotoxic potential against MCF-7 and L6 cells by MTT assay. The qualitative phytochemical screening of the liquid fractions revealed the presence of different secondary metabolites. The quantitative analysis revealed that the liquid fractions were rich in alkaloids, flavonoids and phenolic compounds. Stem bark methanol fraction (SBMF) and leaf methanol fraction (LMF) showed potential cytotoxicity on MCF-7 cells with an  $IC_{50}$  of  $47.11 \pm 3.53 \mu\text{g/ml}$  and  $48.62 \pm 2.40 \mu\text{g/ml}$  respectively. Whereas, stem bark chloroform fraction (SBCF) and leaf chloroform fraction (LCF) showed moderate activity on MCF-7 cells. Vinblastine sulphate was used as a reference standard and it showed potent cytotoxic activity against MCF-7 cells with an  $IC_{50}$  of  $24.03 \pm 2.12 \mu\text{g/ml}$ . Even though Vinblastine is a potent chemotherapeutic drug it affected the viability of normal cells. In comparison with Vinblastine, the liquid fractions showed very less toxicity on normal cells. Hence, the present study suggested that *A. semecarpifolia* stem bark and leaves are the potent cytotoxic agents against MCF-7 cells.

**Keywords:** Alkaloids; *Alseodaphne semecarpifolia*; Cell lines; Cytotoxicity; Flavonoids.

Ethnomedicine is as old as man's history and medicinal plants are being used in ethnomedicine to treat number of ailments. They play a key role in the management of world health<sup>1</sup>. The traditional practices of herbal formulations are the integral part of various medicinal systems before the development of modern science. These herbal remedies are the major sources of traditional medicinal practices for thousands of years<sup>2</sup>.

In the discovery of herbal drugs Indian traditional medicinal plants will be of immense interest for all the ethnopharmacologists who are looking for novel therapeutic approaches to various health problems filled all over the world<sup>3</sup>. It becomes appropriate to search for safe and potent herbal medicines which are already in use by traditional practitioners in order to strengthen their efficacy and safety levels through scientific

validation. The biological activities of medicinal plants are also recognized in pharmaceutical research as the major resources of phytomedicines<sup>4</sup>.

The phytochemical molecules derived from plants have biological activity in humans by having some protective properties against certain disease conditions<sup>5,6</sup>. Hence, there is a great demand to screen bioactive compounds from medicinal plants as a basis for further biomedical investigations. With advanced phytochemical techniques, many active molecules have been screened and developed as potent drugs in modern medicinal systems. The most important secondary metabolites are alkaloids, flavonoids, phenolics and tannin compounds<sup>7,8</sup>. These secondary metabolites and their altered forms are responsible for various therapeutic activities against cancer and other deadly diseases threatening humans all over the world<sup>9</sup>.

Cancer is a disease characterized by gradual deterioration and loss of function in the tissues and organs, associated with the lifestyle and environmental factors. Among different types of cancers breast cancer is a prime global health burden and one of the leading causes of deaths in females<sup>10</sup>. The physical and chemical carcinogens in the environment may induce cell death which may further lead to mutations and cancer causing irreversible damage to DNA. To overcome these risks of cancer, the plant derived anticancer agents play a significant role and many of the modern anticancer drugs are employed in the treatment of various cancers in humans<sup>11</sup>.

*Alseodaphne semecarpifolia* is an evergreen plant commonly known as Nelthare in Kannada<sup>12</sup>, in Western Ghats region of Shivamogga, Karnataka, India it is well known as 'Sehunda'<sup>13</sup>. The *Alseodaphne* species are the potential source of various biologically active compounds. The secondary metabolites of *A. semecarpifolia* are known for their various biological activities<sup>14-16</sup>. The ethno medicinal survey has revealed that the traditional medicinal practitioners in Central Western Ghats of Karnataka, India employed stem bark and leaves of *A. semecarpifolia* in the treatment of human breast cancer. Even though they are employed in the traditional practices for several years, they are lacking sufficient scientific supports. Hence, the present study was initiated aiming to evaluate the traditional medicinal

claims of *Alseodaphne semecarpifolia* as a potent anticancer agent.

## MATERIALS AND METHODS

### Plant sample collection and processing

The stem bark and leaf samples of *A. semecarpifolia* were collected from Karigudda, Central Western Ghats region, Shivamogga, Karnataka, India. The plant was identified and authenticated by Dr. Y. L. Krishnamurthy., Taxonomist, Department of Applied Botany, Kuvempu University, Shivamogga, Karnataka, India. The voucher specimen (KUBPHS78) is deposited in the herbarium of DBT-BUILDER project, Kuvempu University for future reference. The collected stem bark and leaves were washed under running tap water to remove the soil and other dust particles followed by washing with distilled water and blotted. They were air dried in the room temperature to remove the water content. Further, the dried samples were powdered using mechanical grinder and stored in air tight container until the extraction.

### Crude extraction of secondary metabolites

The powdered stem bark and leaf samples were subjected to sequential soxhlet extraction using solvents of increasing polarity i.e. petroleum ether, chloroform and methanol. The extracts were concentrated using rotary evaporator and the solvents were completely evaporated to dryness using water bath and desiccated until further analysis<sup>17,18</sup>.

### Fractionation by partition chromatography

The crude extracts were further fractionated using liquid-liquid partition chromatography. For liquid fractionation 5g of the stem bark and leaf crude methanol extracts were suspended in 100ml of chloroform and centrifuged at 10,000rpm for 10 minutes. The supernatant and residue were collected separately. The fraction collected as a supernatant was considered as chloroform fraction. The fraction collected as a residue was further dissolved in 100ml of methanol and re-suspended with 100ml of hexane in separating funnel. Further it was shaken vigorously for the separation of phytochemical constituents in methanol and hexane. Later the two distinct layers of methanol and hexane were collected separately. The solvents in separated chloroform, methanol and hexane

fractions were dried in water bath and desiccated<sup>19</sup>.

#### **Phytochemical screening of liquid fractions**

Qualitative and quantitative phytochemical analysis of stem bark hexane fraction (SBHF), stem bark chloroform fraction (SBCF), stem bark methanol fraction (SBMF), leaf hexane fraction (LHF), leaf chloroform fraction (LCF) and leaf methanol fraction (LMF) was carried out using the standard procedures with some minor modifications<sup>20-32</sup>. Based on the fractionation yield and presence of phytochemical constituents the chloroform and methanol fractions were selected for cytotoxicity studies.

#### ***In vitro* cytotoxic activity**

##### **Cell culture and maintenance**

The MCF-7 (Breast cancer) and L6 (Normal rat myoblast cells) cells were procured from American Type Culture Collection (ATCC). Dulbecco's Modified Eagle Medium (DMEM) was used to culture the procured stock cells by supplementing with streptomycin, 10% inactivated Fetal Bovine Serum (FBS) and penicillin in a humidified atmosphere of 5% CO<sub>2</sub> at 37°C until the cells were confluent.

##### **Cell seeding and treatment**

MCF-7 and L6 cells were dissociated by using cell dissociating solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The viability of the cells was checked and centrifuged followed by seeding 50,000 cells/well in a 96 well plate. They were allowed to form a monolayer under regular growth conditions followed by treatment with the test samples. The treated and untreated cells were incubated for 24 hours in CO<sub>2</sub> incubator at 37°C with 5% CO<sub>2</sub>, 95% air and 100% relative humidity and these cells were harvested for cytotoxicity studies.

##### **MTT assay**

The cytotoxic effect of SBCF, SBFM, LCF and LMF on MCF-7 and L6 cells was determined by MTT assay<sup>33</sup>. The monolayer cell culture was trypsinized and the cell count was adjusted to 1x10<sup>5</sup> cells/ml using DMEM containing 10% FBS. 100µl of the diluted cell suspension was loaded onto the respective wells in 96 well plate. After incubating for 24 hours, the supernatant was carefully flicked off when the partial monolayer was observed, followed by washing with DMEM. 100µl of different concentrations of the test samples were added to the partial monolayer and the plate was

then incubated at 37°C for 24 hours with 5% CO<sub>2</sub> in CO<sub>2</sub> incubator. After incubation, the test solutions in the wells were discarded and 100µl of MTT (5mg of MTT in 10ml PBS) solution was added. Again the plate was incubated for 4 hours at 37°C with 5% CO<sub>2</sub> in CO<sub>2</sub> incubator. After the incubation, the supernatant was removed and 100µl of dimethyl sulfoxide (DMSO) was added and the plate was gently shaken to solubilize the formed formazan. The absorbance was measured at 590nm using a microplate reader. The IC<sub>50</sub> values were generated from the dose-response curves and the percentage growth inhibition was calculated.

##### **Statistical analysis**

The statistical analysis was performed using GraphPad Prism Software v 5.01 (GraphPad Software Inc., San Diego, CA). The data is presented as mean±SEM of three replicates and it is statistically analyzed using two way analysis of variance (ANOVA) followed by Bonferroni post-test. The p value less than 0.001 was considered statistically significant.

## **RESULTS**

#### **Liquid-liquid fractionation yield**

The liquid-liquid fractionation yield of stem bark hexane fraction (SBHF), stem bark chloroform fraction (SBCF) and stem bark methanol fraction (SBMF) was 0.05g (1%), 0.40g (8%) and 2.87g (57.4%) respectively. The fractionation yield of leaf hexane fraction (LHF), leaf chloroform fraction (LCF) and leaf methanol fraction (LMF) was 0.11g (2.2%), 0.62g (12.4%) and 2.15g (43%) respectively.

#### **Qualitative phytochemical analysis**

The qualitative phytochemical analysis showed that SBHF contains only glycosides and saponins. Alkaloids, glycosides, phenols and terpenoids are the phytochemicals present in SBCF, whereas, SBFM contains alkaloids, flavonoids, glycosides, phenols, saponins and terpenoids. It is noteworthy that flavonoids are present only in SBFM, however, tannins and steroids are absent in all the three stem bark fractions. Similarly, the phytochemical analysis of leaf fractions revealed that LHF contains flavonoids, steroids, glycosides and saponins. LCF showed the presence of alkaloids, steroids, phenols and terpenoids. Whereas, LMF showed to contain

alkaloids, flavonoids, steroids, glycosides, phenols, saponins and terpenoids. It is significant that steroids are present and tannins are absent in all the three leaf fractions. The results of the qualitative phytochemical analysis are presented in Table 1.

#### Quantitative phytochemical analysis

The quantitative phytochemical analysis of liquid fractions revealed the presence of alkaloids, flavonoids, tannins, steroids, glycosides, phenolics, saponins and terpenoids in various concentrations of dry weight of the fractions. SBHF showed the presence of only glycosides and saponins with  $2.09 \pm 0.72 \mu\text{g}/\text{mg}$  and  $10.37 \pm 4.60 \mu\text{g}/\text{mg}$  concentrations respectively. SBCF is rich in Alkaloids ( $26.45 \pm 6.79 \mu\text{g}/\text{mg}$ ) followed by phenolics ( $18.55 \pm 2.86 \mu\text{g}/\text{mg}$ ). Likewise, SBMF is rich in flavonoids ( $110.19 \pm 11.55 \mu\text{g}/\text{mg}$ ) and alkaloids ( $82.45 \pm 8.25 \mu\text{g}/\text{mg}$ ) followed by phenolics ( $50.64 \pm 8.66 \mu\text{g}/\text{mg}$ ) and saponins ( $47.46 \pm 3.39 \mu\text{g}/\text{mg}$ ). The results are presented in Table 2.

LHF contains flavonoids, steroids, glycosides and saponins in very minute quantities, whereas, LCF is rich in alkaloids ( $28.50 \pm 6.84 \mu\text{g}/\text{mg}$ ) and phenolics ( $15.50 \pm 7.92 \mu\text{g}/\text{mg}$ ). However, LMF is very rich in alkaloids ( $108.65 \pm 5.06 \mu\text{g}/\text{mg}$ ) and flavonoids ( $90.73 \pm 7.90 \mu\text{g}/\text{mg}$ ) followed by saponins ( $70.30 \pm 7.67 \mu\text{g}/\text{mg}$ ) and phenolics ( $66.46 \pm 4.06 \mu\text{g}/\text{mg}$ ). The results are presented in Table 3.

#### Cytotoxic effects of stem bark and leaf liquid fractions

The cytotoxic activity of SBCF, SBMF, LCF and LMF was observed in a dose dependent manner. SBMF and LMF showed significant cytotoxic activity on MCF-7 cells with an  $\text{IC}_{50}$  of  $47.11 \pm 3.53 \mu\text{g}/\text{ml}$  and  $48.62 \pm 2.40 \mu\text{g}/\text{ml}$  respectively. However, SBCF and LCF showed moderate activity on MCF-7 cells with an  $\text{IC}_{50}$  of  $116.20 \pm 2.47 \mu\text{g}/\text{ml}$  and  $103.80 \pm 10.74 \mu\text{g}/\text{ml}$  respectively (Fig. 1).

**Table 1.** Qualitative phytochemical analysis of *A. semecarpifolia* stem bark and leaf liquid fractions

| Sl No. | Phytochemical Constituents | Stem bark liquid fractions |      |      | Leaf liquid fractions |     |     |
|--------|----------------------------|----------------------------|------|------|-----------------------|-----|-----|
|        |                            | SBHF                       | SBCF | SBMF | LHF                   | LCF | LMF |
| 01     | Alkaloids                  | -                          | +    | +    | -                     | +   | +   |
| 02     | Flavonoids                 | -                          | -    | +    | +                     | -   | +   |
| 03     | Tannins                    | -                          | -    | -    | -                     | -   | -   |
| 04     | Steroids                   | -                          | -    | -    | +                     | +   | +   |
| 05     | Glycosides                 | +                          | +    | +    | +                     | -   | +   |
| 06     | Phenols                    | -                          | +    | +    | -                     | +   | +   |
| 07     | Saponins                   | +                          | -    | +    | +                     | -   | +   |
| 08     | Terpenoids                 | -                          | +    | +    | -                     | +   | +   |

+: Present; -: Absent

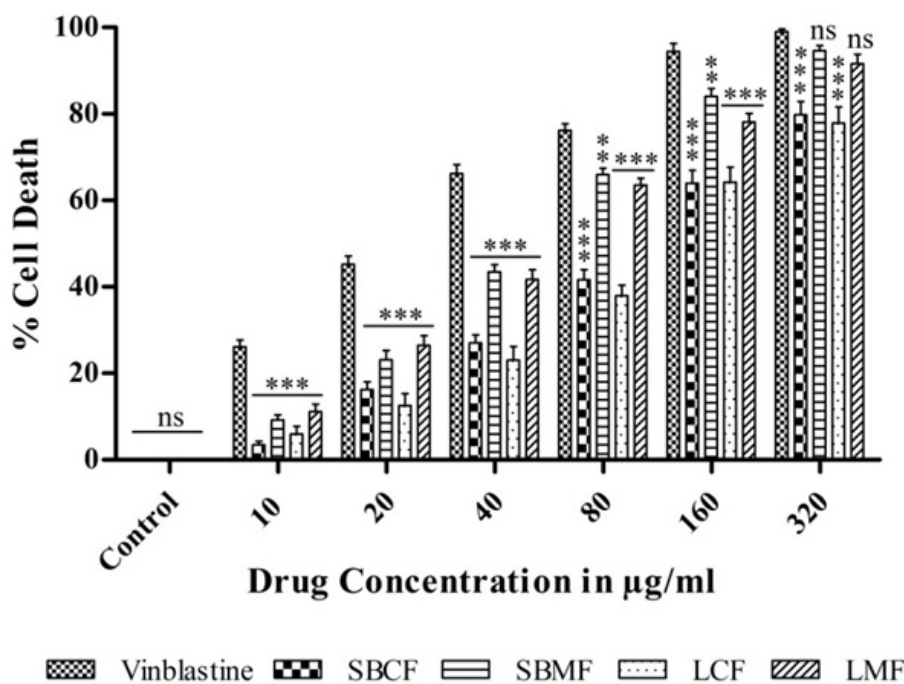
**Table 2.** Quantitative phytochemical analysis of *A. semecarpifolia* stem bark liquid fractions

| Sl No. | Phytochemical constituents | SBHF ( $\mu\text{g}/\text{mg}$ ) | SBCF ( $\mu\text{g}/\text{mg}$ ) | SBMF ( $\mu\text{g}/\text{mg}$ ) |
|--------|----------------------------|----------------------------------|----------------------------------|----------------------------------|
| 01     | Total Alkaloids            | -                                | $16.45 \pm 6.79$                 | $82.45 \pm 8.25$                 |
| 02     | Total Flavonoids           | -                                | -                                | $110.19 \pm 11.55$               |
| 03     | Total Tannins              | -                                | -                                | -                                |
| 04     | Total Steroids             | -                                | -                                | -                                |
| 05     | Total Glycosides           | $2.09 \pm 0.72$                  | $0.48 \pm 1.22$                  | $5.32 \pm 1.01$                  |
| 06     | Total Phenolics            | -                                | $18.55 \pm 2.86$                 | $50.64 \pm 8.66$                 |
| 07     | Total Saponins             | $10.37 \pm 4.60$                 | -                                | $47.46 \pm 3.39$                 |
| 08     | Total Terpenoids           | -                                | $4.09 \pm 3.09$                  | $12.69 \pm 6.70$                 |

The cytotoxic effect of SBCF, SBMF, LCF and LMF was compared with the cytotoxic properties of standard anticancer drug Vinblastine. Vinblastine exhibited significant effect on MCF-7 cells with an  $IC_{50}$  of  $24.03 \pm 2.12 \mu\text{g/ml}$ . Even though Vinblastine exhibited significant effect against MCF-7 cells, it also affected the viability of normal L6 cells with an  $IC_{50}$  of  $88.52 \pm 3.56 \mu\text{g/ml}$ , but SBCF, SBMF, LCF and LMF were very least toxic on L6 cells and their  $IC_{50}$  was unable to determine due to their lesser toxicity (Fig. 2).

### DISCUSSION

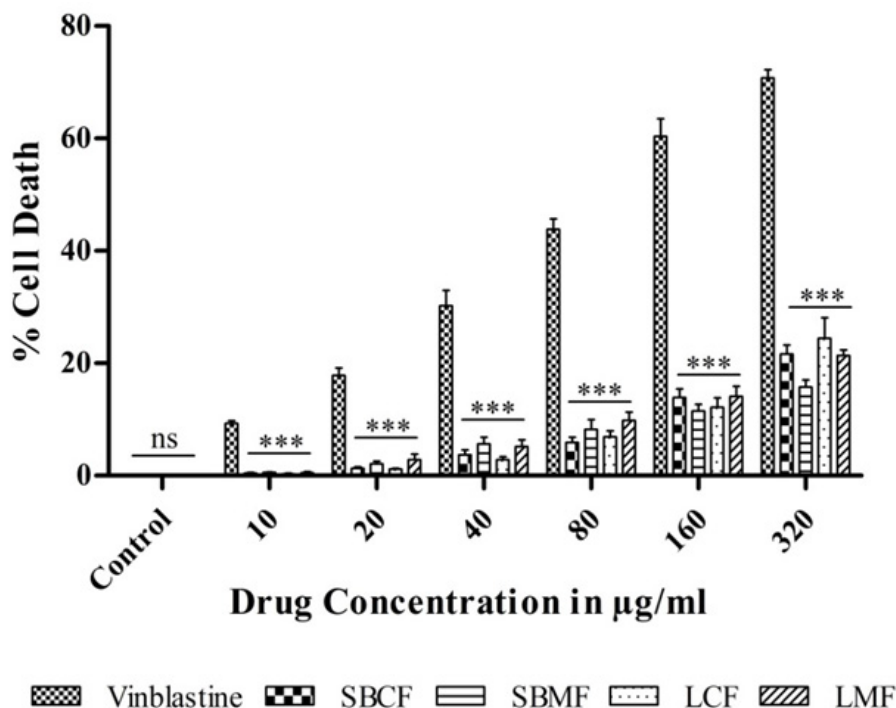
Medicinal plants are believed to be a potent source of secondary metabolites with significant therapeutic properties. The plant secondary metabolites such as alkaloids, flavonoids, saponins, phenols, tannins, terpenoids, glycosides and steroids are found to be important phytochemical constituents<sup>34,35</sup>. Most of the Ayurvedic medicines used today are derived from herbal sources. According to World Health Organization (WHO)



**Fig. 1.** Dose dependent cytotoxic effects of *A. semecarpifolia* stem bark and leaf liquid fractions against MCF-7 cells. ns: non-significant; \*\*: significant; \*\*\*: highly significant

**Table 3.** Quantitative phytochemical analysis of *A. semecarpifolia* leaf liquid fractions

| Sl No. | Phytochemical constituents | LHF (µg/mg) | LCF (µg/mg) | LMF (µg/mg) |
|--------|----------------------------|-------------|-------------|-------------|
| 01     | Total Alkaloids            | -           | 28.50±6.84  | 108.65±5.06 |
| 02     | Total Flavonoids           | 2.61±3.40   | -           | 90.73±7.90  |
| 03     | Total Tannins              | -           | -           | -           |
| 04     | Total Steroids             | 1.48±3.48   | 4.21±8.03   | 19.10±10.04 |
| 05     | Total Glycosides           | 0.66±0.91   | -           | 8.65±7.75   |
| 06     | Total Phenolics            | -           | 15.50±7.92  | 66.46±4.06  |
| 07     | Total Saponins             | 4.62±1.71   | -           | 70.30±7.67  |
| 08     | Total Terpenoids           | -           | 2.09±4.17   | 13.30±5.85  |



**Fig. 2.** Dose dependent cytotoxic effects of *A. semecarpifolia* stem bark and leaf liquid fractions against L6 cells. ns: non-significant; \*\*\*: highly significant

nearly 80% of the human population all over the world is still relying on herbal remedies for preliminary health care. This is due to their availability, lesser side effects and low cost<sup>36,37</sup>. Medicinal plants have the ability to synthesize wide variety of secondary metabolites from their each and every part under different stress conditions. This motivates the researchers all over the world to find novel therapeutic drugs from natural sources with potential biological activities<sup>38</sup>.

Secondary metabolites are not so essential for the normal growth and development of the organisms. Absence of secondary metabolites does not result in immediate death of the organisms, but they have long term impairment on the survivability of the organisms and they often play an important role in protection against certain pathogens and several disease conditions<sup>39,40</sup>. These secondary metabolites function in conjunction with one another or they may act alone in order to bring desired pharmacological effect<sup>41</sup>.

In order to derive the factors involved in anticancer properties, phytochemical screening

was carried out for stem bark and leaf liquid fractions. The extraction of secondary metabolites was carried out sequentially using solvents of increasing polarity i.e. by selecting solvents of three different polarities such as non-polar, medium polar and polar solvents. Non-polar solvents were used to extract out non-polar compounds, whereas the compounds of intermediate polarity were extracted by using medium polarity solvents and polar solvents were used to extract out polar compounds. The sequential extraction allows the preliminary separation of secondary metabolites of distinct polarities which simplifies the further fractionation.

The fractionation of secondary metabolites was carried out using partition chromatography. The molecules need to be separated by Partition chromatography will interact with two immiscible solvents according to the solubility of the compounds<sup>42</sup>. The cytotoxicity of the liquid fractions was evaluated by Microculture Tetrazolium (MTT) assay. Among the different cell viability tests MTT assay is one of the frequently

used technique. It is more advantageous, especially its effectiveness and simplicity makes it more suitable to determine *in vitro* anticancer activities of test drug at the preliminary levels. In this assay the cell viability was determined by using colorimeter<sup>43</sup>.

In the present study, preliminary phytochemical analysis of *A. semecarpifolia* stem bark and leaf fractions have showed the presence of different secondary metabolites which are of great importance in the field of drug research. Different active phytochemicals have been found to possess a wide range of activities, which may help in protection against incurable diseases.

The phytochemical analysis of stem bark and leaf liquid fractions revealed that alkaloids, flavonoids and phenolic compounds are the rich components of *A. semecarpifolia*. This alkaloid, flavonoid and phenolic compounds represent as a most widespread class of bioactive compounds with multiple therapeutic properties<sup>44</sup>. The anticancer property of medicinal plants is due to the presence of alkaloid, flavonoid and phenolic compounds<sup>45-47</sup>. In the present investigation it is evident that SBMF and LMF exerted significant dose dependent cytotoxicity against human breast cancer cells. As alkaloid, flavonoid and phenolic compounds are the rich components of SBMF and LMF they might be responsible for their potent activity. The earlier studies have suggested that *A. semecarpifolia* is a rich source of aporphine alkaloids, isoquinoline alkaloids and phenolic derivatives<sup>48-50</sup>. Among different subgroups of aporphine alkaloids, benzyloquinoline alkaloids form the broad subgroup widely distributed in *Alseodaphne* species<sup>51</sup>.

The cytotoxic properties of SBMF and LMF were compared with the standard anticancer drug Vinblastine. Although Vinblastine shows a potent activity against cancer cells it affected the viability of normal cells. In contrast, the cytotoxic effects of SBMF and LMF on normal cells was very least. To be a potent anticancer drug it should be selectively toxic on cancerous cells but not on non-cancerous cells. By considering all these factors the present study has provided the evidence that SBMF and LMF have promising anticancer property, and supported the traditional medicinal claims of *A. semecarpifolia* as a potent source of anticancer compounds.

## CONCLUSION

Several novel cytotoxic compounds are screened from traditional medicinal plants every year to fight against certain cancers. Even though several natural active compounds have unique anticancer properties, they are not used in clinical practices due to limited bioavailability. On the other hand the secondary metabolites derived from natural sources are the potent leads for drug discovery and development.

The choices of modern drugs for the cancer therapies are limited and most of them are accompanied with dose-related toxicities. The development of an effective chemotherapeutic agent is crucial and new strategies need to be put forth which can help for the identification of natural compounds with superior clinical efficacy and lesser toxicity. It is evident from the present study that, *Alseodaphne semecarpifolia* is a potent anticancer agent against human breast cancer cells.

## ACKNOWLEDGEMENT

This work was financially supported by DBT, New Delhi, India through DBT-BUILDER project (Grant No. BT/PR9128/INF/22/190/2013, Dated: 30/06/2015). The authors are thankful to Skanda Lifesciences Pvt Ltd, Bangalore, Karnataka, India and department of Applied Zoology and Biotechnology, Kuvempu University, Karnataka, India for providing the laboratory facility to carry out this research.

## Conflict of Interest

The authors declare no conflicts of interest for this study.

## REFERENCES

1. Thakkar K, Parmar V, Patel D and Meshram D. Recent Advances in Herbal Drug Standardization - A Review. *Int. J. Adv. Pharm. Res.*, **4**: 2130-2138 (2013).
2. Pandey M. K, Singh G. N, Sharma R and Snehlata. Standardization of Yakrit Plihintak Churna: An Ayurvedic Polyherbal Formulation. *Int. J. Pharm. Sci. Res.*, **3**: 171-176 (2012).
3. Devasagayam T. P. A. Introduction to Serial Reviews: Recent Advances in Indian Herbal Drug Research. *J. Clin. Biochem. Nutr.*, **40**: 73 (2007).

4. Pillai N. R. Antidiarrheal Activity of *Punica granatum* in Experimental Animals. *Int. J. Pharmacogn.*, **30**: 201-204 (1992).
5. Key T. J, Appleby P and Spencer E. A. Cancer Incidence in British Vegetarians. *Br. J. Cancer.*, **101**: 192-197 (2009).
6. Richardson M. A, Sanders T and Palmer J. L. Complementary/Alternative Medicine Use in a Comprehensive Cancer Center and the Implications for Oncology. *J. Clin. Oncol.*, **18**: 2505-2514 (2000).
7. Bekele G and Hazare S. T. Isolation and Characterization of Bioactive Compounds from Medicinal Plants of Ethiopia - A Review. *Current trends in Biomedical Engineering and Biosciences.*, **7**: 1-4 (2017).
8. Li H. B, Jiang Y and Chen F. Separation Methods Used for *Scutellaria baicalensis* Active Components. *J. Chromatogr. B.*, **1**: 277-290 (2004).
9. Thilagavathi T, Arvindganth R, Vidhya D and Dhivya R. Preliminary Phytochemical Screening of Different Solvent Mediated Medicinal Plant Extracts Evaluated. *Int. Res. J. Pharm.*, **6**: 246-248 (2015).
10. Liang C, Pan H, Li H, Zhao Y and Feng Y. *In vitro* Anticancer Activity and Cytotoxicity Screening of Phytochemical Extracts from Selected Traditional Chinese Medicinal Plants. *JBUON.*, **22**: 543-551 (2017).
11. Altobelli E and Lattanzi A. Breast Cancer in European Union: An Update of Screening Programmes as of March 2014 (Review). *Int. J. Oncol.*; **45**: 1785-1792 (2014).
12. Charles A, Joseph M and Ramani A. V. *In vitro* Antioxidant Potential of *Alseodaphne semecarpifolia* Leaf Extract. *Eur. J. Exp. Biol.*, **2**: 354-357 (2012).
13. Kumar A. K. M and Shivaraju H. P. A Study on Traditional Knowledge and Medicinal Applications of the Endemic Herbal Species in the Western Ghats of Shimoga Region, Karnataka, India. *Int. J. Res. Chem. Environ.*, **6**: 1-13 (2016).
14. Charles A and Ramani A. V. Phytochemical Screening and Antimicrobial Resistance of *Alseodaphne semecarpifolia* Nees. *J. Chem. Pharm. Res.*, **3**: 205-211 (2011).
15. Charles A, Joseph M and Ramani A. V. *In vitro* Antioxidant Potential of *Alseodaphne semecarpifolia* Leaf Extract. *Eur. J. Exp. Biol.*, **2**: 354-357 (2012).
16. Puttaramaiah C. G, Venkatarangaiah K and Kakanahalli N. Screening *In vitro* Anticancer Activity of *Alseodaphne semecarpifolia* Nees Stem Bark Extracts against Some Cancer Cell lines. *Pharmacogn. J.*, **11**: 884-888 (2019).
17. Ahmad A, Alkarkhi A. F. M, Hena S and Khim L. H. Extraction, Separation and Identification of Chemical Ingredients of *Elephantopus Scaber* L. Using Factorial Design of Experiment. *Int. J. Chem.*, **1**: 36-49 (2009).
18. Dantu A. S, Shankarguru P, Devi D. R and Hari B. N. V. Evaluation of *In vitro* Anticancer Activity of Hydroalcoholic Extract of *Tabernaemontana divaricata*. *Asian J. Pharm. Clin. Res.*, **5**: 59-61 (2012).
19. Oldoni T. L. C, Cabral I. S. R, d'Arce M. A. B. R, Rosalen P. L, Ikegaki M and Nascimento A. M. Isolation and Analysis of Bioactive Isoflavonoids and Chalcone From A New Type of Brazilian Propolis. *Sep. Purif. Technol.*, **77**: 208-213 (2011).
20. Harborne J. B. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. London: Chapman and Hall; 2005.
21. Edeoga H. O, Okwu D. E and Mbaebie B. O. Phytochemical Constituents of Some Nigerian Medicinal Plants. *Afr. J. Biotechnol.*, **4**: 685-688 (2005).
22. Zhishen J, Mengcheng T and Jianming W. The Determination of Flavonoid Contents in Mulberry and Their Scavenging Effects on Superoxide Radicals. *Food Chem.*, **64**: 555-559 (1999).
23. Senguttuvan J, Paulsamy S and Karthika K. Phytochemical Analysis and Evaluation of Leaf and Root Parts of the Medicinal Herb, *Hypochoeris radicata* L. for *In vitro* Antioxidant Activities. *Asian Pac. J. Trop. Biomed.*, **4**: 359-367 (2014).
24. Siddhuraju P and Becker K. Antioxidant Properties of Various Solvent Extracts of Total Phenolic Constituents from Three Different Agroclimatic Origins of Drumstick Tree (*Moringa oleifera* lam.) Leaves. *J. Agric. Food. Chem.*, **51**: 2144-2155 (2003).
25. Makkar H. P, Siddhuraju P and Becker K. *Methods in Molecular Biology: Plant Secondary Metabolites*. Totowa: Human Press; 2007.
26. Durai M. V, Balamuniappan G, Anandalakshmi R, Geetha S and Kumar N. S. Qualitative and Quantitative Analysis of Phytochemicals in Crude Extract of Big-Leaf Mahogany (*Swietenia macrophylla*). *Int. J. Herb. Med.*, **4**: 88-91 (2016).
27. Singh R, Verma P. K and Singh G. Total Phenolic, Flavonoids and Tannin contents in Different Extracts of *Artemisia absinthium*. *J. Intercult. Ethnopharmacol.*, **1**: 101-104 (2012).
28. Malik S. K, Ahmad M and Khan F. Qualitative and Quantitative Estimation of Terpenoid Contents in Some Important Plants of Punjab,



- Pakistan. *Pak. J. Sci.*, **69**: 150-154 (2017).
29. Indumathi C, Durgadevi G, Nithyavani S and Gayathri P. K. Estimation of Terpenoid Content and its Antimicrobial Property in *Enicostemma littorale*. *Int. J. ChemTech Res.*, **6**: 4264-4267 (2014).
30. Devanaboyina N, Ramalakshmi N, Satyanarayana, Sudeepthi P, Chakradhar K. H and Raju N. P. K. Preliminary Phytochemical Screening, Quantitative Estimation and Evaluation of Antimicrobial Activity of *Alstonia macrophylla* Stem Bark. *International Journal of Science Inventions Today.*, **2**: 31-39 (2013).
31. Ajiboye B. O, Ibukun E. O, Edobor G, Ojo A. O and Onikanni S. A. Qualitative and Quantitative Analysis of Phytochemicals in *Senecio biafrae* Leaf. *Int. J. Invent. Pharm. Sci.*, **1**: 428-432 (2013).
32. El-olemy M. M, Al-muhtadi F. J, and Affi A. F. A. Experimental Phytochemical: A Laboratory Manual, Saudi Arabia: King Saud University Press; 1994.
33. Alley M. C, Scudiere D. A, Monks A, Hursey M. L, Czerwinski M. J and Fine DL, *et al.* Feasibility of Drug Screening with Panels of Human Tumor Cell Lines Using a Microculture Tetrazolium Assay. *Cancer Res.*, **4**: 589-601 (1988).
34. Tiwari P, Kumar B, Kaur M, Kaur G and Kaur H. Phytochemical Screening and Extraction, A Review. *Int. J. Pharm. Sci.*, **1**: 98-106 (2011).
35. Viji M and Murugesan S. Phytochemical Analysis and Antibacterial Activity of Medicinal Plant *Cardiospermum halicacabum* Linn. *J. Phytol.*, **2**: 68-77 (2010).
36. Malar C. G. R and Chellaram C. Phytochemical Screening, Total Flavonoid, Total Terpenoid and Anti-Inflammatory Activity of Aqueous Stem Extract of *Salacia oblonga*. *J. Chem. Pharm. Sci.*, **10**: 550-556 (2017).
37. Murugesan D and Ponnuswamy R. D. Potential Anti-inflammatory Medicinal Plants - A Review. *Int. J. Pharm. Pharm. Sci.*, **6**: 43-49 (2014).
38. Igbinosa O. O, Igbinosa E. O and Aiyegoro A. Antimicrobial Activity and Phytochemical Screening of Stem Bark Extracts from *Jatropacurcas* (Linn). *Afr. J. Pharm. Pharmacol.*, **3**: 58-62 (2009).
39. Thirumurugan D, Cholarajan A, Suresh S. S, Raja and Vijayakumar R. An Introductory Chapter: Secondary Metabolites; 2018.
40. Rehab A, Hussein, Amira A and EI-Anssary. Plant Secondary Metabolites: Key Drivers of the Pharmacological Actions of Medicinal Plants. *J. Pharmacogn. Phytochem.*, **6**: 32-36 (2018).
41. Anand U, Herrera N. J, Altemimi A and Lakhssassi N. A Comprehensive Review on Medicinal Plants as Antimicrobial Therapeutics: Potential Avenues of Biocompatible Drug Discovery. *Metabolites.*, **9**: 1-13 (2019).
42. Ingle P. K, Deshmukh A. G, Padole D. A, Dudhare M. S, Moharil M. P and Khelurkar V. C. Phytochemicals: Extraction Methods, Identification and Detection of Bioactive Compounds from Plant Extracts. *J. Pharmacogn. Phytochem.*, **6**: 32-36 (2017).
43. Mosmann T. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assay. *J immunol methods.*, **65**: 55-63 (1983).
44. Stevigny C, Bailly C and Quetin-Leclercq J. Cytotoxic and Antitumor Potentialities of Aporphinoid Alkaloids. *Curr Med Chem-Anticancer Agents.*; **5**: 173-182 (2005).
45. El-Ansari M. A, Ibrahim L. F and Sharaf M. Natural Phenolics: A Source of Anticancer Agents. *Egypt. Pharm. J.*, **18**: 1-7 (2019).
46. Isah T. Anticancer Alkaloids from Trees: Development Into Drugs. *Pharmacogn. Rev.*, **10**: 1-11 (2016).
47. Ahmed S. I, Hayat M. Q, Tahir M, Mansoor Q, Ismail M and Keck K. Pharmacologically Active Flavonoids From The Anticancer, Antioxidant And Antimicrobial Extracts of *Cassia angustifolia* Vahl. *BMC Complementary Altern. Med.*, **16**: 460 (2016).
48. Charles A, Stanly L. A, Joseph M and Ramani A. V. GC-MS Analysis of Bioactive Components on the Bark Extract of *Alseodaphne semecarpifolia* Nees (Lauraceae). *Asian J. Plant Sci. Res.*, **1**: 25-32 (2011).
49. Charles A, Joseph M and Ramani A. V. Phytochemical Analysis of *Alseodaphne semecarpifolia* Leaf Extract by GC-MS. *Asian J. Pharm. Clin. Res.*, **6**: 89-92 (2013).
50. Thakur B. K, Anthwal A, Rawat D. S, Rawat B, Rashmi and Rawat M. S. M. A Review on Genus *Alseodaphne*: Phytochemistry and Pharmacology. *Mini-Rev. Org. Chem.*, **9**: 433-445 (2012).
51. Mukhtar M. R, Zahari A, Nafiah M. A, Hadi A, Thomas A. H and Hiroko N. F. 3', 4'-Dihydrostephasubine, A New Bisbenzylisoquinoline from the Bark of *Alseodaphne corneri*. *Heterocycles.*, **78**: 2571-2578 (2009).