



ANTICANCER ACTIVITY OF *Pedaliium murex* Linn METHANOLIC LEAVES EXTRACT AGAINST A549 HUMAN LUNG CANCER CELL LINE

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AUTHORS' CONTRIBUTIONS

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ABSTRACT

Pedaliium murex (Pedaliaceae), commonly called Large Caltrops, is understood for its pharmacological uses in traditional medicine system. It is reported to possess excellent medicinal properties that helps cure reproductive disorders, mainly impotency in men, nocturnal emissions, gonorrhoea also as leucorrhoea in women. Apart from that, it also contains remedy for urinary and gastrointestinal tract disorders. Pharmacologically, the plant has been explored for antiulcerogenic, nephroprotective, hypolipidemic, sexual enhancer, mitigating, antidermatophytic, cancer prevention agent, antimicrobial and insecticidal exercises. The present review may be a bundle of data collected from the published research articles and highlights the phytochemical and pharmacological aspects of *P. murex*. Highly presences of methnolic extract are information will be helpful in developing the new formulation with therapeutic and economical value in the future. A549 cells treated with *Pedaliium murex* leaves methanolic extract in different hours (6, 12, 24 and 36 hours) after the 36 hours the cells growth are controlled.

Keywords: Human cancer cell line A549; inorganic elements; phytomedicine; *Pedaliium murex*.

1. INTRODUCTION

Cancer may be a generic term for an outsized group of diseases characterized by the expansion of abnormal cells beyond their usual boundaries which will then invade adjoining parts of the body and spread to other organs. Normally, human cells grow and divide to make new cells because the body needs them. When

cells get older or become damaged, they die, and new cells take their place. While cancer develops, though, this orderly process breaks down. The particular process becomes imbalanced and therefore the cells start dividing no end and should form growths called tumors (Fig. 1). Cancerous tumors are malignant, which suggests they will spread into, or invade, nearby tissues and even visit foreign places within the

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body through the blood and lymph. Unlike malignant tumors, benign tumors don't spread into, or invade, nearby tissues. Benign tumors can sometimes be quite large, however. Nearly every family within the world is touched by cancer, which is now liable for almost one in six deaths globally.

Cancer may be a group of diseases characterized by the uncontrolled growth of cells which will cause death. Despite many therapeutic treatments available for cancer, the survival rate and disease curative percentage are very low. It's become an increasing public ill health that accounts for six million cases per annum throughout the planet [1]. The varied sorts of cancer, carcinoma is that the second most vital explanation for death worldwide that accounts for 75e80% of the deaths. Current cancer chemotherapy can damage or kill the rapidly dividing and healthy cells, which causes serious side effects like anaemia, hair loss, and nausea. Additionally, the prices of chemotherapeutic drugs are high compared to those of the natural compounds derived from medicinal plants. Therefore, the utilization of natural products might be an alternate method to regulate and eradicate cancer. Natural sources like plants, microorganisms and marine organisms are potential bioresources for anticancer compounds [2]. The infusion prepared from the plant may be a highly prized remedy among the people of Southern India within the treatment of gonorrhoea and dysuria. It's also given as a remedy for spermatorrhoea, enuresis and impotency [3]. *Pedaliium murex* are often used as herbal medicine is belonging to the Pedaliaceae. It's a cooling tonic, purifies blood, act as diuretic and removes stone from the bladder Antioxidant rich plant extract function a source of nutraceuticals that alleviate the oxidative stress and thus prevent or hamper the degenerative diseases. An attempt has been made to explore the antiproliferative activity of fruit extracts of *P. murex* [4] from the natural yield, like plant extracts or pure compounds, there's an opportunities for the

event of latest drug thanks to its availability of chemical diversity. Therefore, researchers are gradually turning back their attention to traditional medicine, for adaptation of best drug against microbial infections. Nowadays, nearly 80% of peoples round the world use plant as source for drugs either directly or indirectly particularly in developing countries [5,6]. Traditional medicine has a improved and developing countries as an exchange solution to health problems and expenses of pharmaceutical products [7]. Phytochemical analysis of persons plants revealed that some plants are shows potential source of some chemical constituents like alkaloids, flavonoids, glycosides, phenols and antioxidants with radical scavenging activity [8]. Polyphenolic compounds are secondary plant metabolites found in numerous plant species and that they are reported to possess multiple functions to counteract the free radicals and that they also inhibit differing types of oxidizing enzymes [9]. Free radicals are often either harmful or helpful to the body. When there's an imbalance within the formation and removal of free radicals, then a condition called as oxidative stress is developed within the body. To counteract these free radicals, the body has protective antioxidant mechanisms with the skills to lower incidence of varied human morbidities and mortalities [10]. Since the dawn of human evolution, medicinal plants are used for the health and burly relationship with the human society. Among several diseases within the world, infectious diseases is imperative diseases encountered by clinicians in developing nations from that tract infections (UTIs) is extremely serious ill health about 57 millions peoples were expire per annum. Thanks to its toxic effects and multi drug resistance of microbial pathogens, the effectiveness of medicine get reduced for that there's a rise attempt by researchers to seek out new drugs from medicinal plants supported available antibiotics developed by pharmacological industries [11].

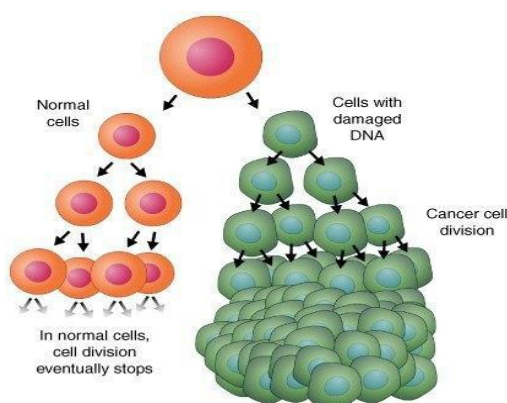


Fig. 1. Cell multiplication in cancer cells and normal cell

However, many researchers suggest that the production of these substances have lack of quality, in trade and prescription therefore it is more important to focus in the standardization and treatment to the peoples. Based on the survey with the herbal drug sellers, local tribal peoples and ethanopharmacologists the plant *P. murex* has been used frequently for the treatment of various diseases in human and animals. Lot of other medicinal uses of this plant are pains, nervine weakness, Indigestion, Renal calculi, Inflammation, Piles, Constipation, Cough, Infections, Epitasis, Heart related problems, Asthma, Frigidity, Impotence. The purpose of the present study was to explore out the phytochemical content and possible antimicrobial agents against urinary tract infection bacteria using different extracts of plant *P. murex* treat for uncurable diseases without any complicated side effects. The presence of phenolic and flavonoids compounds in the plant will improve the antioxidants capacity of this plant and it serve as natural antioxidant against diseases in human. The phenolic compounds are the central ingredients of dietary applications and it includes polyphenols (hydrolyzable and condensed tannins), phenolic acids (hydroxybenzoic and hydroxycinnamic acids) and flavonoids it has the properties to reduce the coronary heart disease, death due to cancer and protective in several health-related properties. Free radicals are extremely active atoms or molecules with unbalancing valence electron which takes part in human body by the process of energy creating, immunization, the signaling and the cell growth. Further, it may cause various cell and tissue disorders with damage or alteration of DNA structure as a result it leads to cell fatality, abnormal in a gene, cancer, heart attack, atherosclerosis, diabetes, malaria, hypertension, rheumatoid arthritis, cancer and neurodegenerative disorder and it is treated with the help of plant containing curative properties [12,13]. Some diseases like ulcer, dysuria, splenic enlargement, diarrhea, gonorrhoeal rheumatism, aphrodisiac and demulcent were treated by using leaves. The seed of this plant was used as a treatment of leucorrhoea, urinary tract disorder, joint pain, lumbago, bladder troubles and gonorrhoea. Using stem parts of *P. murex* used for the treatment of spermatorrhoea, dysuria, ardour urinae and gonorrhoea [14].

2. MATERIALS AND METHODS

2.1 Plant Collection

The fresh leaves of *Pedaliium murex* were collected from Perumalagaram village, Thiruvarur District, Tamil Nadu, India.

2.2 Plant Material

The *Pedaliium murex* leaves was dried under shade, mechanically reduced to a moderately coarse powder, and stored in amber-colored airtight containers. The coarse type of the medication was utilized for the assurance of physicochemical boundaries like dampness content, debris esteems, expanding file, frothing record, unfamiliar natural issue, extractive qualities, fluorescence analysis.

2.3 Preliminary Phytochemicals Screening

Preliminary phytochemical screening was performed using standard procedures [15-17]. The concentrates acquired from various solvents were exposed to distinguishing proof tests for the location of different phytoconstituents through natural and inorganic components examination, by means of the strategy [18].

2.4 Culturing of Cell Lines

The Vero and human lung cancer cells (A549) were obtained from Kings Institute of Preventive Medicine and Research, Guindy, Chennai. The cells were grown in 96-well tissue culture (TC) plate in Dulbecco's Minimum Essential Medium (MEM) with Trypsin-phosphate-verseneglucose (TPVG) solution, 10% New Born Calf Serum (NBCS) (Gibco-Invitrogen), 100 U/mL of penicillin (Gibco-Invitrogen) and 100 µg/mL of streptomycin (Gibco-Invitrogen). The cells were incubated in CO₂ incubator (Haier Electric Co., Ltd.) at 37°C in 95% humidified atmosphere enriched by 5% CO₂ and sub-cultured every 3-4 days once.

2.5 MTT Cell Viability Assays

The MTT assay was done using the methodology of Siddiqui et al. (2010). The monolayer of cell culture was trypsinized and the cell count was adjusted to 1.0x10⁵ cells/ml using growth medium. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10, 000 cells /well) was added. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, the monolayer was washed once and 100 µl of leaves of *Pedaliium murex* methanolic extract with different concentrations (100, 200, 250, 500 and 1000 µg/ml) was added to each well. The plates were then incubated at 37°C for 3 days in 5% CO₂ atmosphere, and microscopically examined at the end of 6, 12, 24 and 36 hours for recording the result. After 72 hours, the test solutions in the wells were discarded and 50 µl of MTT in HBSS-PR was added to each well. The

plates were gently shaken and incubated for 3 hours at 37°C in 5% CO₂ atmosphere. The supernatant was removed and 50 µl of propanol was added and therefore the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at the wavelength of 540 nm. The percentage growth inhibition was calculated using the formula given below:

$$\% \text{ Cytotoxicity} = (1 - \text{Abs test} / \text{Abs Control}) \times 100$$

2.6 Statistical Analysis

Statistical analysis was performed by one way Analysis of Variance (ANOVA) followed by Duncan's multiple range test (DMRT) using Software Package for the Social Science (SPSS) software package version 15.00. Results were expressed as Mean ± Standard Deviation for p values <0.05 were considered significant for analysis of percent inhibition of cell growth.

3. RESULTS AND DISCUSSION

Plant have given a wellsprings of motivation to novel medication mixes as plant inferred meds have made critical commitment towards human wellbeing, phytomedicines can be utilized for the treatment of disease as done in case of Unani and Ayurvedic system of medicines or it can be the base for the development of a medicine, a natural blue print for the development of a drug. Phytochemicals are referred to as phytonutrients. These are compounds present in plant derived-foods that induce biological activities in the body. They have various saluvrious functions in the body. For instance, in this way phytonutrients advance the capacity of the invulnerable framework, act directly against microscopic organisms and infections, diminishes aggravation and are additionally connected with the treatment and anticipation of cancer, cardiovascular disease and many other maladies affecting the health or well being of an individual. Plant biochemistry is epitomize the considerable diversity of organic substances that are intricate and accumulated through plants, the chemical composition of these substances, their biosynthesis turn over and metabolism in plants, their innate circulation and their biological medicinal plants are supportive for therapeutic as well as for remedial of human diseases, since of the occurrence of phytochemical constituents [19].

3.1 Cytotoxicity Activity of *Pedaliium murex*

The *Pedaliium murex leaves* methanolic extract was initially evaluated for their cytotoxic effects on vero (Normal) cell lines. The maximum cytotoxicity (20%)

of methanolic extract was observed at 1000 µg/ml concentration.

3.2 Anticancer Activity on A549 Cell Lines

The *Pedaliium murex leaves* methanolic extract showed the anticancer activity against A549 cell line in a dose dependant manner. A549 cells treated with *Pedaliium murex leaves* methanolic extract in different hours (6, 12, 24 and 36 hours) after the 36 hours the cells growth are controlled. The *Pedaliium murex leaves* methanolic extract showed a maximum inhibition of 68% at 500 µg/ml against A549 lung cancer cells. The present investigation the minimum inhibition of 12% was recorded at 100 µg/ml concentration. The *Pedaliium murex leaves* methanolic extract showed the anticancer activity against A549 cell line in a dose dependant manner. A549 cells treated *Pedaliium murex leaves* methanolic extract in different concentration level (100, 200, 250, 500 and 1000 µg/ml) after the 36 hours the cells growth are changes occurred. A549 cell normally has a polygonal shape and sheet like pattern in normal monolayer culture, which is compatible with its epithelial origin. The *Pedaliium murex leaves* methanolic extract showed a maximum inhibition of 68% at 500 µg/ml against A549 lung cancer cells. The present investigation the minimum inhibition of 12% was recorded at 50 µg/ml concentration. Herbal mediated silver nanoparticles playing important role to create eco-friendly, cost effective and stable nanoparticles. The researches on synthesis of silver nanoparticles using various plant extracts found that it is safer and better in cancer treatment, but more plants are still not explored for the synthesis of nanoparticles and its applications in pharmaceutical industries. For the most part chemotherapy, medical procedure and radiation treatment are the most common remedial alternative for malignant growth Sadly, these medicines have different reactions because of absence of focused conveyance. Synthesis of herbal mediated silver nanoparticles provides controlled and targeting action of drug, which can also overcome the problems associated with conventional cancer treatments [14].

Natural products in simplest term are the chemical compounds, produced by living organisms. The cells of living organisms can be considered as micro chemical reactors producing large number of chemical compounds through metabolic reactions. Ayurvedic system of medicine in India and is used in curing various diseases like stomach disorders, gastric ulcers and to stimulate appetite. Saponins are a diverse group of compounds widely distributed in the plant kingdom. Consumer demand for natural products coupled with their physicochemical properties and mounting evidence on their biological activity such as

anticancer and anticholesterol activity has led to the emergence of saponins as commercially significant compounds with expanding applications in food, cosmetics, and pharmaceutical sectors. The present study has been formulated to understand the phytochemical screening, *in vitro* antioxidant and anticancer properties elicited by saponin from roots of *Decalepis hamiltonii*. The cytotoxic properties were evaluated, on cancer cells of Breast cancer(MCF-7), lung cancer (A-549) cell lines and compare with normal cell line L-6 (Rat, Normal muscle) using MTT colorimetric assay for 24, 48 and 72 hrs. There is no systematic work that has been undertaken on this plant and this is the first report of the phytochemical screening, antioxidant and anticancer activities elicited by saponin from *Decalepis hamiltonii* [20].

The herbal extracts have antimicrobial, antioxidant properties which have been studied widely. The herbal extracts of peppermint such as methanol, ethanol and aqueous has flavonoids. It is responsible for the anti-cancer activity of a compound. The cytotoxicity of the compounds were tested in A549, lung cancer cell line and compared with L132, normal embryonic lung cell line by MTT assay. The cell viability test shows that the compounds has toxic effect against A549 cell line and has negligible toxicity against L132 cell line [21]. The pharmaceutical industry develops the drugs with the consideration of important characteristics such as solubility, membrane permeability, metabolic stability and systemic pharmacokinetics and pharmacodynamics.

Table 1. Qualitative analysis of *Pedaliium murex* leaves extract

S. no	Analysed phytochemicals factor	Methanol	Ethanol	Water
1.	Tannin	++	+	+
2.	Phlobatannins	-	+	+
3.	Saponin	+	+	-
4.	Flavonoids	++	+	-
5.	Steroids	++	-	+
6.	Terpenoids	+	+	+
7.	Triterpenoids	+	+	-
8.	Alkaloids	++	+	+
9.	Carbohydrate	+	-	+
10.	Protein	++	-	+
11.	Anthraquinone	+	-	+
12.	Polyphenol	++	+	+
13.	Glycoside	+	-	-

Indications: “+” means positive activity, “-” means negative activity
Tannin, Phlobatannins, Saponin, Flavonoids, Steroids, Terpenoids, Triterpenoids,
Alkaloids, Carbohydrate, Protein, Anthraquinone, Polyphenol and Glycoside

Table 2. Qualitative analysis of inorganic elements analysis of *Pedaliium murex* leaves extract

S. no	Inorganic elements	Result
1.	Calcium	+
2.	Magnesium	+
3.	Sodium	+
4.	Potassium	++
5.	Iron	++
6.	Sulphate	+
7.	Phosphate	+
8.	Chloride	+
9.	Nitrate	+

Indications: “+” means positive activity, “-” means negative activity
Pedaliium murex leaves extract powdered is the main source of good quality source of pharmacognostical supplementation of this *Pedaliium murex* leaves extract may be useful for human health associated emerging diseases such as diabetes, hypertension and cancer

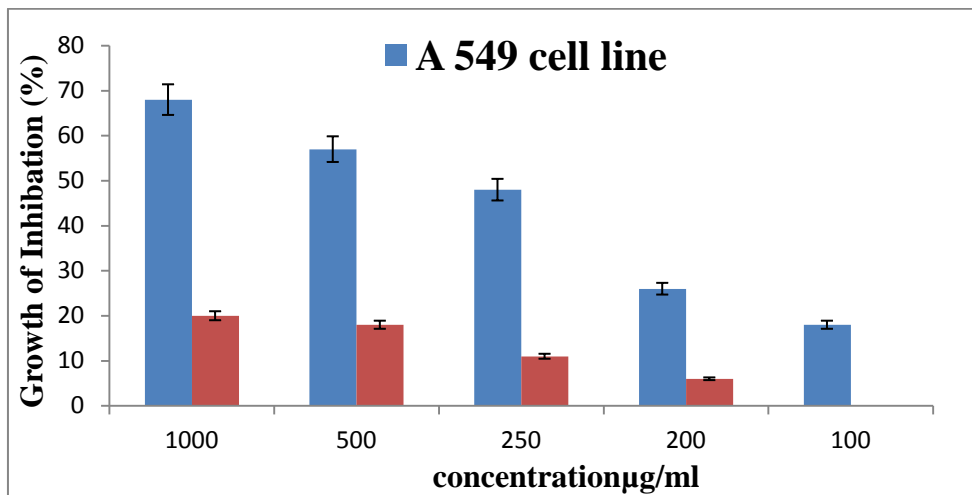


Fig. 2. Compared anticancer activity on normal Vero cell line to A549 cell lines in different concentration

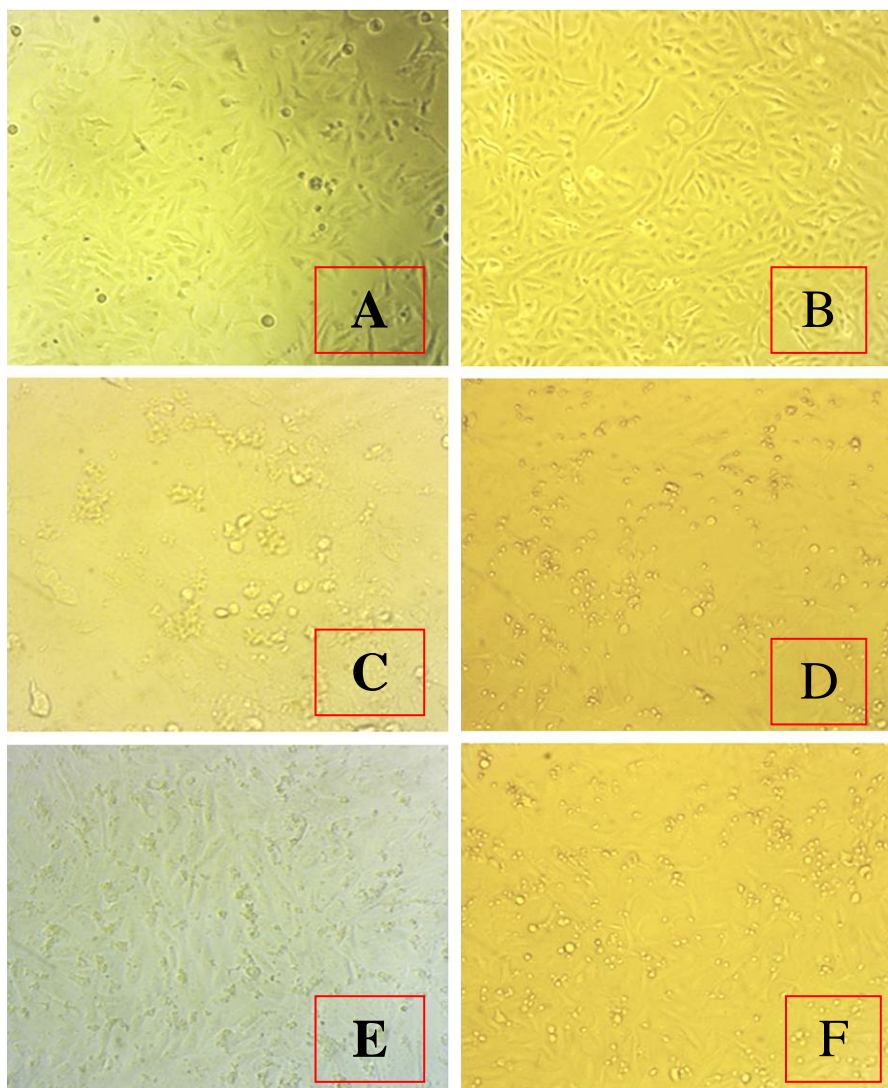


Fig. 3. A549 lung cancer cell culture in different concentration
A. control, B. 100 µg/ml, C. 200 µg/ml, D. 250 µg/ml, E. 500 µg/ml, F. 1000 µg/ml

4. CONCLUSION

Pharmacological and phytochemical studies done so far on *P. murex* confirm the claims of traditional uses of the plant. Based on the literature, it can be concluded that it has a better profile with the potential to serve as a natural source for the treatment of various ailments. Being rich in antioxidant, it can serve as a source of nutraceuticals that could alleviate the oxidative stress and therefore can be used to prevent or slow down the degenerative diseases. It can also be used as a source of pesticide of plant origin to combat blast disease of rice in the field by formulating the compound as a biopesticide. However, pharmacological and phytochemical studies have been carried out independently and lack identification of isolation of active molecules. Therefore, more research is required to correlate its pharmacological activity with chemistry based evidences by strengthening linkage between researches being carried out by different groups across the world so that it can be developed as potential drugs.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Hakkim FL, Al-Buloshi M, Al-Sabahi J. Frankincense derived heavy terpene cocktail boosting breast cancer cell (MDA-MB- 231) death *in vitro*. Asian Pac J Trop Biomed. 2015;5(10):824e828.
- Cheng YL, Lee SC, Lin SZ, Chang WL, Chen YL, Tsai NM, Liu YC, Tzao C, Yu DS, Harn HJ. Anti-proliferative activity of *Bupleurum scrozonrifolium* in A549 human lung cancer cells *in vitro* and *in vivo*. Cancer Lett. 2005;222(2):183e193.
- Patel DK, Kumar R, Prasad SK, Hemlatha S. *Pedaliium murex* Linn (Pedaliaceae) fruits: A comparative antioxidant activity of its different fractions. Asian Pac J Trop Biomed. 2011;1(5):395–400.
- Singh NP, Panda H. Medicinal herbs with their formulation, Daya Publishing House, New Delhi, India. 2005;671.
- WHO. Traditional Medicine: Growing Need and potentials WHO policy perspectives on medicines. World Health Organization Geneva. 2002;1-6.
- Saidu Talatu Bosso, Daniel Augustine Innalegwu. Phytochemical and antibacterial activity of methanol extract of *Garcinia kola* against selected bacteria isolated. American Journal of Food, Nutrition and Health. 2018;3(1):26-30.
- Riazunnisa K, Adilakshamma U, Habeeb Khadri C. Phytochemical analysis and *in vitro* antibacterial activity of *Soymida febrifuga* (Roxb.) Juss. and *Hemidesmus indicus* (L.). Ind. J. App. Res. 2013;3:57-59.
- Tikadar P, Palita SK, Panda D. Phytochemical analysis of medicinal plants used for treatment of dysentery and diarrhoea by the Paraja Tribe of Koraput, Odisha, India. Int. J. Herb. Med. 2017;5:1-4.
- Rezaeizadeh A, Zuki ABZ, Abdollahi M. Determination of antioxidant activity in methanolic and chloroformic extracts of *Momordica charantia*. Afr. J. Biotechnol. 2011;10:4932-4940.
- Shinde A, Ganu J, Naik P. Effect of free radicals and antioxidants on oxidative stress. J. Dent. Allied Sci. 2012;1:63-66.
- Tauseef Shaikh, Rukhsana A. Rub, Sasikumar S. Antimicrobial screening of *Cichorium intybus* seed extracts. Arabian Journal of Chemistry. 2016;9:S1569–S1573.
- Badakhshan Mahdi-Pour, Subramanion L. Jothy, Lachimanan Yoga Latha, Yeng Chen, Sreenivasan Sasidharan. Antioxidant activity of methanol extracts of different parts of *Lantana camara*. Asian Pac J Trop Biomed. 2012;2(12):960-965.
- Efstathia Skotti, Eirini Anastasaki, Georgia Kanellou, Moschos Polissiou, Petros A. Tarantilis. Total phenolic content, antioxidant activity and toxicity of aqueous extracts from selected Greek medicinal and aromatic plants. Industrial Crops and Products. 2014;53:46–54.
- Muhammad Imran, Naresh Kumar, Ferozuddin Nohri, Dileep Kumar, Tayyuba Kousar, Muhammad Tauseef Sultan, Sajjad Ali Ilyas, Shabnam Shahida. Phytochemical and pharmacological potentials of *Pedaliium murex* Linn and its traditional medicinal uses. Journal of Coastal Life Medicine. 2015;3(9):737-743.
- Khandelwal KR. Practical pharmacognosy techniques and experiments. New Delhi: Nirali Prakashan. 2002;15-163.

16. Kokate CK. Practical pharmacognosy. 1st Ed. New Delhi: Vallabh Prakashan. 2005;111.
17. British Pharmacopoeia. 1st Edition, TSO Publisher, London, United Kingdom. 2010;5000.
18. The Government of India, Ministry of Health and Family Welfare. The Ayurvedic Pharmacopoeia of India Vol-3, Part-1. 1st Ed. New Delhi: Ministry of Health and Family Welfare. 2001;233-251.
19. Alagendran S, Fernandez-Saavedra G, Pushpa N, Sahaya Sathish S, Sathish P, Valarmathi M, Sudha P, Mohanlal VA, Vijay N. Int J. Life Sci Pharma Res. 2020;SP-07. ISSN: 2250 – 0480. International Conference on Cancer Research.
20. Dinesh Ram DS, Vijayakumar N, Rubalakshmi G. Int J Life Sci Pharma Res. 2020;SP-07. ISSN: 2250 – 0480. International Conference on Cancer Research.
21. Pennarasi Mohanraj, Akbarsha Mohammad Abdulkader, Suthira Selvam, Thirumurugan Ramasamy. Int J Life Sci Pharma Res. 2020;SP-07. ISSN: 2250 – 0480. International Conference on Cancer Research.