Research Article

Major weed *Parthenium hysterophorus* L. as a propitious plant with its free radical scavenging activity and anticancer activity against A549 lung cancer cell line

Jagadish Kumar Suluvoy, Ebin Philip Thompson, V. M. Berlin Grace*

ABSTRACT

Objective: The objective of this study was to investigate the antioxidant and anticancer activity of the *Parthenium hysterophorus* L. against A549 lung cancer cell line. **Background:** *P. hysterophorus* L. is a pernicious herb which is a weed with dominating nature in the productive crop field, by collecting minerals and water before the productive crop takes. *P. hysterophorus* L. belongs to the family of Asteraceae and grows energetically even in low water supply in all fields and gardens. Many recent research findings have shown that the weeds are having immense medicinal potency including anticancer activity. **Materials and Methods:** In this study, the methanolic extract of the leaves of P. hysterophorus L. was analyzed for the presence of various biological active phytochemicals through gas chromatography–mass spectrometry (GC-MS) analysis and its antioxidant activity. The *P. hysterophorus* L. was also analyzed for its anticancer activity against A549 cancer cell line. **Outcome Measure:** The *P. hysterophorus* L. weed which can be grown easily can be used for the human beneficial and the discovery of the new therapeutic drugs. **Results:** The GC-MS analysis has shown the presence of betahistine, hexadecanoic acid methyl ester, phytol, benzaldehyde, 4 (dimethylamino), pentadecanoic acid, 14-methyl-, and methyl ester which are biologically active compounds. The methanolic extract of the *P. hysterophorus* L. was tested for the free radical scavenging activity by doing 2,2-diphenyl-1-picrylhydrazyl assay and its inhibitory concentration (IC50) value was found to be 122.85 µg/ml. The anticancer activity was analyzed by the 3-(4,5-dimethyl-2-thiazolyl)2,5-diphenyl-2,4 tetrazolium bromide assay against A549 lung cancer cell line. An increase in cancer cell growth inhibition was observed with an increase in the concentration of the drug and its IC50 value is 73.74 µg/ml. **Conclusion:** From the study results, it is evident that the weed *P. hysterophorus* L. is a rich source of phytochemicals as shown by GC-MS report which can be used in different medication for the human welfare. This *P. hysterophorus* L. is found to have a good antioxidant property as well as a promising anticancer effect.

KEYWORDS: 2,2-Diphenyl-1-picrylhydrazyl, A549, Asteraceae, Gas chromatography–mass spectrometry, 3-(4,5-Dimethyl-2-Thiazolyl)2,5-diphenyl-2,4 Tetrazolium Bromide, *Parthenium hysterophorus* L.

INTRODUCTION

Weeds are the redundant material which grows in the agriculture crop fields which compete with the useful crop fields by dominating and bringing down the yield of the crop fields.[1] Granting all these, these weeds are having intense uses in enriching the soil, vegetative cover, protect soil from the erosion, and nutrient recycling, and these weeds add organic matter to the soil both from the roots and above the ground level.[2] In a study, 8000 plants were compared with the weeds for their biological compounds of medicinal values than the other plants. Furthermore, 119 therapeutic pharmaceutical industries worked on 101 plant species and found that 36 weed plants are with more biological active compounds.[3] About 50% of drugs sold by the pharmaceuticals in 1991 were dependent on the plant products and its derivatives for the preparation of medicine.[4] Among all these weed plants, *Parthenium hysterophorus* L.[5] is a major weed, which grows near crop fields without uptake of more water and is noticed as unwanted waste plant now.

Free radicals or the ions which possess unpaired single electron with highly reactive nature tend to react with the DNA and proteins to disturb the normal function of the human body.[6] Reactive oxygen species (ROS) and reactive nitrogen species (RNS)

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Department of Biosciences and Technology, Karunya Institute of Technology and Sciences, Coimbatore, Tamil Nadu, India

***Corresponding author:** V. M. Berlin Grace, Department of Biosciences and Technology, Karunya Institute of Technology and Sciences, Coimbatore - 641 114, Tamil Nadu, India. E-mail: berlinbiochem@gmail.com

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are the usual free radicals and are produced during the cellular metabolism. These ROS and RNS have both beneficial and pernicious role in the body. However, more harmful side effects result in the body when ROS and RNS are increased.[7] The free radicals are harmful when they are produced in large quantities when less production of antioxidants occurs in the body. The free radicals that enter the body from the environment also will damage the normal metabolic function of the DNA and cellular lipids.[8] The free radicals lead to the oxidative stress in the cell and disrupt the cell biomolecules. It was reported that such disruption of the cells by free radicals prevents the apoptosis and it develops into a cancer cell.[9] The plants are the natural source of antioxidants, which can scavenge the free radicals and eliminate the stress caused by the free radicals in the body.[10] These phytochemicals are responsible for the antioxidant activity and have the capabilities for scavenging the free radicals. The plant extract with the phytochemicals will help in fighting against cancer (anticancer agent).[11]

The *P. hysterophorus* L. root and flower are rich with phytochemicals and have exhibited antioxidant potential with lipo-protective activity against membrane damage.[12] The aerial parts of the plant were used as a tonic for the emmenagogue and antidysenteric.[13] It is reported that the *P. hysterophorus* L. is having antibacterial activity against pathogenic organisms[14] and it is used as antitumor agent.[15] The profitable nature of the weeds made us to work further on the *P. hysterophorus* L. weed for its phytochemicals, antioxidant, and anticancer properties.

MATERIALS AND METHODS

Plant Sample Collection

The aerial parts of the plant material were collected from the surroundings of Karunya University, Coimbatore, and were taxonomically authenticated by the Botanical Survey of India (BSI), Coimbatore, Tamil Nadu. The plant sample was identified as *P. hysterophorus* L. and the authentication not given by the BSI is BSI/SRC/5/23/2017/Tech/1492.

Chemicals and Reagents

All the chemicals and reagents used for the experiments were of analytical grade and molecular grade procured from the Hi-Media, Sigma, and SD Fine Chemicals.

Extraction

The aerial parts of the *P. hysterophorus* L. were collected, rinsed under tap water followed by distilled water to remove the dust, and allowed to dry under shade. After complete drying, the plant sample was powdered in a blender and extraction process was carried out in a Soxhlet apparatus using three different solvents (methanol, ethanol, and petroleum ether). After extraction, the remaining solvent was evaporated by rotary evaporator and the extract is stored in freezer for further experimental use.

Cell Line

A549 cell line is the human adenocarcinoma human (Lung) alveolar basal epithelial cells procured from the National Centre for Cell Science, Pune. The cells were subcultured with the RPMI1640 media supplemented with 10% FBS and maintained in a humidified atmosphere of 5% CO_2 with 37°C incubator for further use.

Phytochemical Screening

The crude extracts procured by the methanol, ethanol, and petroleum ether were used for examining the presence of phytochemicals, and the qualitative results were expressed as (+) for the presence and (-) for the absence of the phytochemicals. The solvent which extracted more phytochemicals was used for the further analysis of gas chromatography–mass spectrometry (GC-MS), antioxidants, and anticancer property.

Test for Phenols

The test extract was treated with four drops of alcoholic FeCl3 solution. Formation of bluish-black color indicates the presence of phenols.[16]

Test for Flavonoid

Shinoda test

To the dry extract (2 g), 5 ml of ethanol was added along with five drops of hydrochloric acid. 0.5 g of magnesium turnings were added. The presence of flavonoids can be indicated by the appearance of pink color.[17]

Test for Tannins

The distilled water is used to mix the plant extract and filtered, and a few drops of 5% ferric chloride were added to the filtrate slowly and the blue–green color indicates the presence of tannins.[16]

Test for Saponins

Each of the plant extract (0.5 g) was separately shaken with distilled water (10 ml) in a test tube. After vigorous shaking, the foam formation indicates the presence of saponins.[16]

Test for Alkaloids

Iodine and potassium iodide were mixed in distilled water, and a few drops of this solution were added to the extract dissolved in distilled water. The brown-colored precipitates indicate the presence of alkaloids.[17]

Test for Steroids and Triterpenoids

The leaf extract was mixed with chloroform, and a few drops of acetic anhydride were added to the solution.

This above solution was boiled in a water bath and rapidly cooled in ice water. Formation of a brown ring after adding concentrated H_2SO_4 to the cooled solution at the junction of the two layers and the appearance of the green color indicate the presence of steroids.[17]

Test for Glycosides

Keller-Killiani test

The extract was mixed properly with distilled water and glacial acetic acid containing a few drops of ferric chloride was added, followed by H_2SO_4 along the sides of the test tube. The formation of brown ring at the interphase gives a positive indication for cardiac glycoside.[18]

Test for Terpenoids

Salkowski test

The crude extract (about 100 mg) was separately shaken with chloroform (2 ml) followed by the addition of concentrated H_2SO_4 (2 ml) along the sides of the test tube, and a reddish brown coloration at the interphase indicates the presence of terpenoids.[18]

GC-MS Analysis

The methanolic extract of *P. hysterophorus* L. was analyzed using GC-MS for the presence of biologically important compounds. The thermo GC-trace ultra VER: 5.0 (Bremen, Germany) and MS MSDSQ II electron mode with 70 eV ionization energy were used. The column temperature was set at about 70–260°C at the rate of 6°C/min. The GC injector was set at a temperature of 280°C and MS transfer at 290°C, respectively. The helium gas was used as carrier gas with a flow rate of 1.0 ml/min, and 1 µl of the sample was used for the analysis. The major compounds that are present in the leaf extract were analyzed by the retention time and the mass fragmentation patterns. The National Institute of Standards and Technology (NIST) and Wiley 2.0 library were used for the detection of the compounds.[19]

Free Radical Scavenging Activity by 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Method

The free radical scavenging capacity was carried out for the methanolic extract of different concentrations. 0.1 mM solution of DPPH in methanol was prepared, and ascorbic acid was used as standard. Series of standard in the range of 5–200 µg/mL and leaf sample in the range of 25–200 µg/mL were prepared. Each sample was made up to 3 mL with distilled water and 1 mL of DPPH was added, and the samples were kept incubation for 30 min and the absorbance was measured at 517 nm. The percentage of scavenging activity of both standards and samples was calculated using the formulae given below, and the graphs were plotted. From these graphs, inhibitory concentration (IC50) values were calculated for the standards and extracts.[20]

Formulae: % inhibition = {A0–A1/A0}*100

A0: Absorbance of control

A1: Absorbance in the presence of the extract

3-(4,5-Dimethyl-2-Thiazolyl)2,5-diphenyl-2,4 Tetrazolium Bromide (MTT) Assay

The MTT assay is based on the metabolic activities of viable cells, the yellow soluble tetrazolium salt. MTT was reduced by succinate dehydrogenase present in the mitochondria of metabolically active cells into water-insoluble purple formazan crystals. These intracellular formazan crystals can be solubilized by dimethyl sulfoxide (DMSO) or isopropanol. 0.1 ml of the cells was seeded on a 96-well plate with 80% confluency and incubated at 37°C for 24 h in the CO_2 incubator. After incubation, the methanolic extract of *P. hysterophorus* L. leaves at different concentrations (25, 50, 75 µg, 100, 125, and 150 µg) was added onto A549 cell line and again incubated for 24 h.[21] MTT (5 mg/ml) was added to each well (10 µl), and the plate was incubated for 4 h. At the end, the entire reaction was arrested by adding 30 µl of DMSO. At 570 nm using an ELISA plate reader, the optical density (OD) values are noted and the inhibition of the cells were calculated by the formula:

$$\text{Percentage of cell viability:} \quad \frac{\text{Mean OD of untreated cells (control)} - \text{Mean OD of treated cells}}{\text{Mean OD of untreated cells (control)}} \times 100$$

RESULTS

Phytochemical Analysis

The *P. hysterophorus* L. leaf powder was dried and extracted phytochemicals using different solvents (ethanol, methanol, and petroleum ether). The methanolic extract of the *P. hysterophorus* L. has shown more phytochemicals when compared to other solvent extracts. Hence, further assays were carried out using the methanolic extract of *P. hysterophorus* L. leaves [Table 1].

The methanolic extract of the *P. hysterophorus* L. showed more phytochemicals than the other solvents.

GC-MS Results

The *P. hysterophorus* L. methanolic extract was tested for the phytochemical compounds by GC-MS analysis. The NIST and Wiley 9.0 library were used to identify the compounds by its relative abundance and time [Figure 1]. The compounds with more than 25% probability are shown in Table 2. By this GC-

MS report, betahistine, hexadecanoic acid methyl ester, phytol, benzaldehyde, 4 (dimethylamino), pentadecanoic acid, 14-methyl-, and methyl ester were identified which are having therapeutic effects and are beneficial for humans [Table 3].

Free Radical Scavenging Activity by DPPH Method

The *P. hysterophorus* L. leaf extract showed an increased scavenging activity of DPPH radicals with the increase in the concentration of the leaf extract. Ascorbic acid was used as the standard for determining the IC50 value. A decrease in the OD values was observed with the increase in the concentration of the leaf extract. The percentage of inhibition and regression curve for standard ascorbic acid and the leaf extract was plotted. The IC50 value of *P. hysterophorus* L. leaf extract and standard ascorbic acid was found to be 122.85 µg/ml and 89.11 µg/ml, respectively [Table 4].

The increasing concentration of the *P. hysterophorus* L. leaf extract showed increasing inhibition activity, and it was compared with the standard ascorbic acid. The IC50 value of the *P. hysterophorus* L. and ascorbic acid is 122.85 µg/ml and 89.11 µg/ml, respectively [Figure 2].

Table 1: The phytochemicals of the *Parthenium hysterophorus* L. in different solvent extracts

Phytochemicals	Ethanol	Methanol	Petroleum ether
Phenol	+	+	+
Flavonoid	-	+	-
Tanins	+	+	-
Saponins	-	-	-
Alkaloids	-	+	-
Steroids	+	+	+
Glycosides	+	+	+
Terpinoids	+	+	-
Triterpinoids	+	+	+

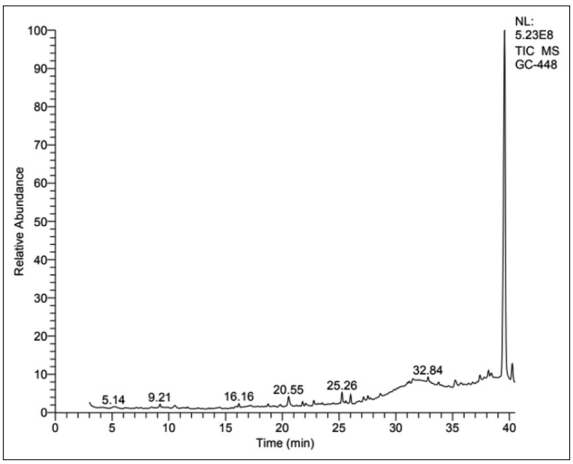

Figure 1: Chromatogram of the gas chromatography–mass spectrometry analysis for the *Parthenium hysterophorus* L. extract

Figure 2 shows linear curve between concentration and inhibition, and the increased concentration of the *P. hysterophorus* L. leaf extract showed the increased percentage of inhibition.

Anticancer Activity by MTT Assay

The methanolic extract of *P. hysterophorus* L. leaves was analyzed for the anticancer property against A549 cancer cell line by the help of MTT assay. The increase in the concentration of the plant extract showed increased cell death in the MTT assay. The IC50 value of the methanolic *P. hysterophorus* L. extract was found to be 73.74 µg/ml [Table 5].

The increased concentrations (25, 50, 75, 100, 125, and 150 µg) of the P. hysterophorus L. showed increased inhibition of the A549 cancer cells and its IC50 value is 73.74 µg/ml [Figure 3].

The increased concentration of the *P. hysterophorus* L. showed increased inhibition of the A549 cancer cells and its IC50 value is 73.74 µg/ml.

DISCUSSION

The metabolites of plants were divided into two major constituents known as primary constituents and secondary constituents. This primary constituents help the plant in growth and reproduction, whereas the secondary constituents would not participate in these processes. However, the secondary constituents which indirectly help the plant for its growth and development are called secondary metabolites.[22] These secondary metabolites are having intense scavenging activity against free radicals (antioxidant activity), and they can be used as antioxidants to fight against free radicals.[23] Impotency of the antioxidants in the body will help the free radicals to develop tumor clones. The cells in the tumor area escape the process of apoptosis, leading to established cancer growth. The *P. hysterophorus* L. is a weed which grows abundantly in crop fields and was not recognized due to its weed nature. However, in this, we evaluated the *P. hysterophorus* L. for the antioxidant nature by its phytochemicals availability. The phytochemicals were extracted using ethanol, methanol, and petroleum ether from the leaves of *P. hysterophorus* L. The methanolic extract of the plant extracted more phytochemicals except saponins, i.e., phenols, flavonoids, alkaloids, terpenoids, triterpenoids, glycosides, and steroids which were present.

In the methanolic extract of the *P. hysterophorus* L., the betahistine, hexadecanoic acid methyl ester, phytol, benzaldehyde, 4 (dimethylamino), pentadecanoic acid, 14-methyl-, and methyl ester were identified in the GC-MS report. All these compounds were having the biological properties such as betahistine,[24] hexadecanoic acid methyl ester,[25] phytol,[26] benzaldehyde,[27] 4 (dimethylamino), pentadecanoic acid, 14-methyl-, and methyl ester (PubMed) which can be used to improve human life. This phytochemicals are

Table 2: Compounds with more than 25% probability in GC-MS report for *Parthenium hysterophorus* L.

Compound name	Molecular formula	Molecular weight	% Probability
N-(2-Bromo-2,4 dimethyl-3 pentylidene) isopropylamine	$C_{10}H_{20}BrN$	233	26.91
[(4-methyl-7-p-methoxyphenyl-ü(3)-cyclopent-2-enyl)(cyclopentadiene) (dicarbonyl)]molybdenium	$C_{22}H_{24}MO_3$	434	54.83
3,3-Bis (4-hydroxy-3,5 dimethylphenyl) pentanedioic Acid Diethyl Ester	$C_{25}H_{32}O_6$	428	43.09
1-(2-trimethylsiloxyvinyl)-4-trimethylsiloxy-3,5-dideuteriobenzene	$C_{14}H_{22}D2O_2Si2$	280	53.13
4-O-Benzyl-6-O-(à-methoxybenzyl)-myo-inositol Orthoformate	$C_{22}H_{24}O_7$	400	80.31
2,6-bis[(3' Methoxyphenyl) sulfanyl] pyrimidine	$C_{18}H_{16}N_2O_{22}$	356	34.97
Pentadecanoic acid, 14-methyl-, methyl ester (CAS)	$C_{17}H_{34}O_2$	270	43.36
Hexadecanoic acid, methyl ester (CAS)	$C_{17}H_{34}O_2$	270	41.68
Hexadecanoic acid	$C_{16}H_{32}O_2$	256	55.33
Phytol	C20H40O	296	54.32
9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-(CAS)	$C_{19}H_{32}O_2$	292	47.12
4,4-dimethyl-2 oxabicyclo[3.2.1]octan-3-one	$C_9H_{14}O_2$	154	29.78
Glycerol 1-palmitate	$C_{19}H_{38}O_4$	330	25.27
Hexadecanoic acid, 2,3-dihydroxypropyl ester (CAS)	$C_{19}H_{38}O_4$	330	25.27
Benzaldehyde, 4(dimethylamino)- (CAS)	$C_9H_{11}NO$	149	27.88
1-Deuterio-1-cyclopropyl-3-methyl-1,2-butadiene	$C_8H_{11}D$	108	29.7
Betahistine	$C_8H_{12}N_2$	136	40.37
5'-[2'-(Cyanomethylene) hydrazino] thieno[2',3':2,3]-5à-cholestan-4'-carbonitrile	$C_{33}H_{45}N_5S$	543	27.61
6-(t-Butylimino)-8-(3'-trifluoromethylphenyl)-3,4-dihydro-2H, 6H-pyrimido[2,1-b][1,3]thiazine-7-carbonitrile	$C_{19}H_{19}F_3N_4S$	392	59.13

GC-MS: Gas chromatography–mass spectrometry

Table 3: Compounds identified in GC-MS report for *Parthenium hysterophorus* L. with biological activity

Compound	Activity
Betahistine	Treatment of vertigo[24]
Hexadecanoic acid, ethyl ester	Antioxidant, hypocholesterolemicnematicide[25]
Phytol	Antimycobacterial[26]
Benzaldehyde, 4(dimethylamino)	Anti-microbial activity[27]
Pentadecanoic acid	Major part of milk [Pubmed]

GC-MS: Gas chromatography–mass spectrometry

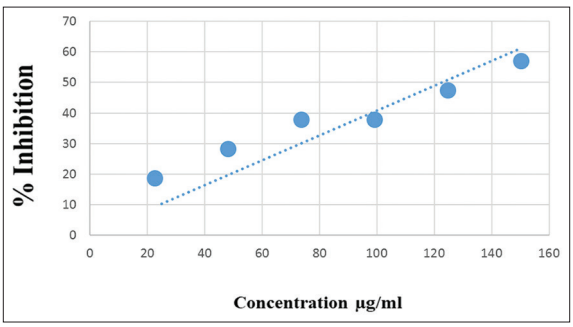

Figure 2: The 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity of the *Parthenium hysterophorus* L.

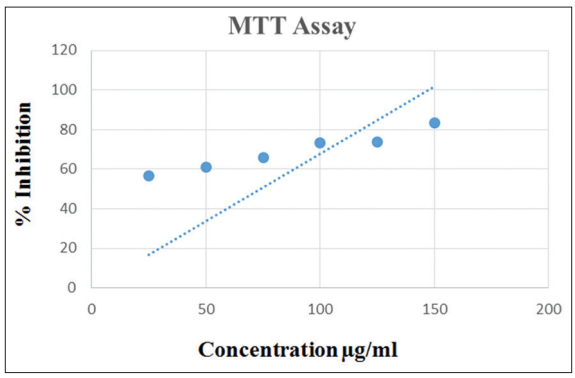

Figure 3: The graph of 3-(4,5-Dimethyl-2-Thiazolyl)2,5-diphenyl-2,4 Tetrazolium Bromide assay report for the *Parthenium hysterophorus* L. against A549 cancer cell lines

the compounds responsible for the antioxidant nature, which scavenge the free radicals. The DPPH activity of the *P. hysterophorus* L. leaf extract showed a good antioxidant activity with an IC50 value of 122.85 µg/ml. It explains that the *P. hysterophorus* L. leaf extract is having highly potent phytochemicals with antioxidant nature. Cancer development is a multistep process, spreading from one place to other in the body. The increase in the number of free radicals in body leads to the endogenous DNA breaks, and this DNA breakage induces mutations in the body. This mutation leads to the formation of cancer and this free radicals are the important class of carcinogens which cause cancer.[7]

The inability of the antioxidants in the body also leads to the adverse effects of carcinogenesis. To overcome this, the plants are the best source for curing cancer by eliminating the free radicals and to induce the apoptosis. In our study, the MTT assay was carried out for the A549 human cell line, and the different concentrations of the 25, 50, 75, 100, 125, and 150 µg inhibited the cell growth with an IC 50 value of 73.74, indicating the potent anticancer effect of *P. hysterophorus* L. leaves against human lung cancer cell lines.

Table 4: DPPH Free radical scavenging activity of the *Parthenium hysterophorus* L. leaf extract

Concentration µg/ml	Average OD	% of Inhibition
25	0.153±0.006	15
50	0.138±0.006	23.3
75	0.127±0.004	29.44
100	0.11667±0.005	35.4
125	0.096±0.005	46.66
150	0.085±0.006	52.77

MTT: 3-(4,5-Dimethyl-2-Thiazolyl)2,5-diphenyl-2,4 Tetrazolium Bromide

Table 5: The anti-cancer activity of the *Parthenium hysterophorus* L. at different concentrations

Concentration µg/ml	Average OD value	Inhibition
25	0.212±0.011	56.91
50	0.191±0.007	61.17
75	0.167±0.007	66.05
100	0.130±0.018	73.57
125	0.113±0.009	77.03
150	0.080±0.007	83.63

CONCLUSION

The enormous weeds in the nature which grow at any circumstances, with very less care, can be used for the human beneficial. The *P. hysterophorus* L. leaves found to have major phytochemicals, which explain the antioxidant nature of the leaf extract in this study. The *P. hysterophorus* L. also showed an anticancer activity against A549 cancer cell line. The weed *P. hysterophorus* L. can be, thus, used further for the identification of major compounds, separation, and its pharmacological importance for human use.

REFERENCES

1. Rahman AH, Islam AK, Rahman MM. The Family *Asteraceae* of Rajshahi Division, Bangladesh. Germany: VDM Verlag Dr. Muller Publishing House Ltd.; 2011.
2. Henkel A. Weeds Used in Medicine. Vol. 188. Washington, DC: US Dept. of Agriculture; 1904.
3. Stepp JR. The role of weeds as sources of pharmaceuticals. J Ethnopharmacol 2004;92:163-6.
4. Neill MJ, Lewis JA. The renaissance of plant research in the pharmaceutical industry. In: Kinghorn AD, Balandrin MF, editors. Human Medicinal Agents from Plants. Washington, DC: American Chemical Society; 1993. p. 48-55.
5. Patel S. Harmful and beneficial aspects of *Parthenium hysterophorus*: An update. 3 Biotech 2011;1:1-9.
6. Liou GY, Storz P. Reactive oxygen species in cancer. Free Radic Res 2010;44:479-96.
7. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J, *et al*. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007;39:44-84.
8. Ridnour LA, Isenberg JS, Espey MG, Thomas DD, Roberts DD, Wink DA, *et al*. Nitric oxide regulates angiogenesis through a functional switch involving thrombospondin-1. Proc Natl Acad Sci U S A 2005;102:13147-52.
9. Dreher D, Junod AF. Role of oxygen free radicals in cancer development. Eur J Cancer 1996;32A:30-8.
10. Xu DP, Li Y, Meng X, Zhou T, Zhou Y, Zheng J, *et al*. Natural antioxidants in foods and medicinal plants: Extraction, assessment and resources. Int J Mol Sci 2017;18:96.
11. Kwon KH, Barve A, Yu S, Huang MT, Kong AN. Cancer chemoprevention by phytochemicals: Potential molecular targets, biomarkers and animal models. Acta Pharmacol Sin 2007;28:1409-21.
12. Kumar S, Mishra A, Pandey AK. Antioxidant mediated protective effect of *Parthenium hysterophorus* against oxidative damage using *in vitro* models. BMC Complement Altern Med 2013;13:120.
13. Oudhia P. Medicinal uses of congress weed *Parthenium hysterophorus* L.: A review. Ecol Environ Conserv 2001;7:175-7.
14. Pandey AK. Anti-staphylococcal activity of a pan-tropical aggressive and obnoxious weed *Parthenium hysterophorus*: An *in vitro* study. Natl Acad Sci Lett 2007;30:383.
15. Reddy DM, Qazi NA, Sawant SD, Bandey AH, Srinivas J, Shankar M, *et al*. Design and synthesis of spiro derivatives of parthenin as novel anti-cancer agents. Eur J Med Chem 2011;46:3210-7.
16. Banso A, Adeyemo S. Phytochemical screening and antimicrobial assessment of *Abutilon mauritianum*, *Bacopa monnifera* and *Datura stramonium*. BIOKEMISTRI 2006;18:39-44.
17. Joshi A, Bhobe M, Saatarkar A. Phytochemical investigation of the roots of *Grewia microcos* Linn. J Chem Pharm Res 2013;5:80-7.
18. Ayoola GA, Coker HA, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, *et al*. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in South Western Nigeria. Trop J Pharm Res 2008;7:1019-24.
19. Suluvoy JK, Berlin Grace VM. Phytochemical profile and free radical nitric oxide (NO) scavenging activity of *Averrhoa bilimbi* L. Fruit extract. 3 Biotech 2017;7:85.
20. Abdul-Wahab NZ, Shahar S, Abdullah-Sani H, Pihie AH, Ibrahim N. Antioxidant, antibacterial and antiviral properties of *Goniothalamus umbrosus* leaves methanolic extract. Afr J Microbiol Res 2011;5:3138-43.
21. Kumar SJ, Shaji S, Grace VB. *Anti-lymphoma* activity of Averrhoa bilimbi fruit extract in Swiss Albino Mice. Asian J Pharm 2016;10:S183.
22. Krishnaiah D, Sarbatly R, Bono A. Phytochemical antioxidants for health and medicine a move towards nature. Biotechnol Mol Biol Rev 2007;2:97-104.
23. McCall MR, Frei B. Can antioxidant vitamins materially reduce oxidative damage in humans? Free Radic Biol Med 1999;26:1034-53.
24. Lacour M, Sterkers O. Histamine and betahistine in the treatment of vertigo: Elucidation of mechanisms of action. CNS Drugs 2001;15:853-70.
25. Kumar PP, Kumaravel S, Lalitha C. Screening of antioxidant activity, total phenolics and GC–MS study of *Vitex negundo*. Afr J Biochem Res 2010;4:191-5.
26. Cantrell CL, Franzblau SG, Fischer NH. Antimycobacterial plant terpenoids. Planta Med 2001;67:685-94.
27. Adegoke OA. Analytical, biochemical and synthetic applications of para-dimethylaminobenzaldehyde. Synthesis 2011;11:4.

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