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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING, INVITRO ANTIOXIDANT AND CYTOTOXIC STUDIES ON *WALTHERIA INDICA* LINN

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ABSTRACT

The current investigation deals with the detection of class of compounds present, free radical scavenging activity and cytotoxicity, of the stem and leaves of Waltheria indica. For this studies Petroleum ether, Chloroform and Methanol were used for extraction. Free radical scavenging activity was evaluated by using the DPPH, (1,1-diphenyl-2-picryl hydrazyl radical),FRAP(Ferric reducing ability of plasma)and LPO(Lipid peroxidation inhibitory activity) methods and Cytotoxicity was measured by MTT assay on cancer and normal cell lines. Among the three plant extracts, Methanol extract showed stronger IC_{50} values in DPPH method (IC_{50} of <62.5), Petroleum ether showed stronger IC_{50} values in LPO method (IC_{50} is 233± 3.9). Among the extracts methanol showed the stronger reducing power with higher absorbance in FRAP method (absorbance ranging 0.524 – 0.272).For all the methods concentrations are maintained from 62.5 to 1000µg/ml, and all the extracts showed the dose dependent activity. The extracts also showed cytotoxity towards normal (Vero) and cancer cell lines (HaCaT, A549, HeLa, HT-29) when evaluated by MTT assay. A panel of 62.5 to 1000 µg/ml concentrations are maintained and concentration of extract needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves. Of the three extracts chloroform extract showed highest % of inhibition at concentration of 1000µg/ml (% of inhibition is 83.86) and is CTC_{50} 103.33±5.77) followed by petroleum ether extract. (CTC_{50} is 133.33±5.8).

INTRODUCTION

Herbal drugs referred as plants materials or herbals, involves the use of whole plants or parts of plants, to treat injuries or illnesses. Herbal drugs are use of therapeutic herbs to prevent and treat diseases and ailments or to support health and healine. These are drugs or preparations made from a plant or plants and used for any of such purposes. Herbal drugs are the oldest form of health care known to mankind. There are many herbal products offered that assert to treat the symptoms of a broad range of problems, from depression to cold and flu World. Health Organization (WHO) has distinct herbal drugs as complete, labeled medicinal products that have vigorous ingredients, aerial or secretive parts of the plant or other plant material or combinations. In India, Ayurveda medicine has used many herbs such as turmeric possibly as early as 1900 BC. Earliest Sanskrit writings such as the Rig Veda, and Atharva Veda are some of the earliest available documents detailing the medical knowledge that formed the basis of the Ayurveda system. Many other herbs and minerals used in Ayurveda were later described by ancient Indian herbalists such as Charaka and Sushtuta during the 1st millennium BC.

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The Sushruta Samhita attributed to Sushruta in the 6th century BC describes 700 medicinal plants, 64 preparations from mineral sources, and 57 preparations based on animal sources.
It is well accepted that reactive oxygen species (ROS), such as superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\cdot}$) formed *in vivo* are highly reactive chemical species and can be generated endogenously as well as exogenously. Excess production of ROS leads to oxidative stress, which can cause number of diseases. In such conditions dietary intake of antioxidant compounds are needed in assisting the body to neutralize the free radicals to remove the harmful effects of oxidative stress. There is an increasing trend to replace synthetic antioxidants, which are of safety concern, with the natural antioxidants available from plant extracts or isolated products of plant origin. Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods. Antioxidant activities are due to the presence of flavones, isoflavones, flavonoids, anthocyanin, catechins and isocatechins. Antioxidants are phyto chemicals, vitamins and nutrients that protect our cell from damage caused by free radicals. They can be found in most fruits vegetables and medicinal herbs. Herbal drugs have gained importance in recent years because of the efficiency and cost effectiveness. Cancer is a general term applied to a series of malignant diseases which may affect many different parts of the body.

If the process is not arrested, it may progress until it causes the death of the organism (Evans, 2002).

Cancer is one of the major causes of death in developed countries, together with cardiac and cerebrovascular diseases (Ueda *et al*., 2002). According to the American Cancer Society, on an average, 559,312 people die of the disease each year (http://www.cancer.org/docroot/STT/stt_o_2008.asp?s, 2008) despite tremendous efforts to find methods of control and cure. Thus, not surprisingly, every fourth citizen of a developed country will be stricken sometime during his/her life and approximately 400 new incidents emerge per 100,000 people annually (Spiridon and Maria, 2004). The statistics released by WHO in 2013 indicate that there is a high likelihood of developing countries approaching the same incident rates of cancer as developed ones, because of life style changes, average age of the population, tobacco usage, etc (Okwu, 2005; Pal, 2007). In a scenario, where conventional medicine has failed to develop techniques that could reduce the incidence of death due to cancer, complementary and alternative medicine (CAM) is slowly emerging as an option. A variety of ingredients of traditional medicines and herbs are being widely investigated in several parts of the world to analyse their potential as therapeutic agents (World Health Organisation, 2007). Drug discovery from medicinal plants has played an important role in the treatment of cancer and, indeed, most new clinical applications of plant secondary metabolites and their derivatives over the last half century have been applied toward combating cancer (http://www.cancer.org/ docroot/STT/ stto _2008.asp?s, 2008; Spiridon and Maria, 2004) *W. indica* L. or sleepy morning, also known as velvet leaf, marsh-mallow, monkey bush, boater bush, leather coat, buff coat, and many other names (Burkill, 2000) belong to the family Sterculiaceae. It is found throughout the tropics and warmer subtropics of India.

The plant has been used as an infusion or decoction where febrifugal, purgative, emollient, tonic, analgesic and astringent action is sought (Burkill, 2000). In some places it is used to make herb tea. The plant produces a fiber that was formerly used for making cords, sacking, padding and sandals. Stems are used as a chew stick; extracts of the plant are used for treatment of cough and curing female sterility. The root is chewed to relieve sore throat as well astreatment of gonorrhoea and leprosy in humans. Stems are used as a chew stick while its extracts are used as an eye bath and a remedy for hemoptysis, treatment of cough and a cure for female sterility (Wagner *et al*., 1990). From literature search, there is a lack of scientific proof to support the ethnomedicinal importance of this plant in the treatment of diarrhea, dysentery, pulmonary troubles, venereal diseases, diabetes,cancer,etc.., Hence, this study was designed to investigate the qualitative phytochemical components of *W. indica* and determine the antioxidant and cytotoxic activities of the crude chloroform, methanol and petroleum ether extracts by using DPPH, LPO and FRAP as antioxidant methods and MTT assay for cytotoxic studies.

MATERIALS AND METHODS

Plant collection and Extraction

The Plant materials were collected from different locations of southern India during the month april 2014. *Waltheria indica* (Tirumala, Andhra Pradesh India), Toddalia asiatica. (Tirupathi, Andhra Pradesh. India). The plants were confirmed by comparing with the housed authenticated specimens. The collected plant materials were shade dried and powdered sample was extracted with methanol, chloroform and petroleum ether successively with soxhlet apparatus, the extracted materials were dried under reduced pressure.

Media and Chemicals

2,2-Diphenyl 1- picryl solution (DPPH),Dimethyl sulfoxide (DMSO), Potassium ferricyanide,10% trichloroacetic acid, TBA, 3-(4,5–dimethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide (MTT), Trypan blue, were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, glucose and antibiotics from Hi-Media Laboratories Ltd Mumbai. Dimethyl Sulfoxide (DMSO) and Propanol from Merck Ltd, Mumbai, India.

Phytochemical Screening

Different qualitative chemical tests were performed for establishing the profile of given extract for its chemical composition. The following tests were performed using standard protocols on the extracts to detect various phyto constituents present in them

Detection for carbohydrates (Rosenthaler, 1930)

500 mg of extract was dissolved in 5 ml of distilled water and filtered. The filtrate was used to test the presence of carbohydrates.

Molisch's test

Molish reagent

10 gm of alpha napthol was dissolved in 100 ml of 95% methanol to prepare Molish reagent. To the extract, two drops of Molish reagent and few drops of concentrated H_2SO_4 is added, formation of purple-violet ring indicates the presence of carbohydrates.

Detection of Glycosides (Ronsenthaler 1930; Middeltone, 1956)

0.5 gm of the extract was hydrolyzed with 20 ml of HCl (0.1 N) and filtered. The filterate was used to test the presence of Glycosides.

Keller-Killiani test

To the extract, few drops of glacial acetic acid and one drop of 5% $FeCl_3$ and concentrated H_2SO_4 was added, formation of reddish brown colour at the junction of two liquid layers and upper layer turned bluish green indicates the presence of glycosides.

Detection of Saponins (Kokate, 2001)

Foam test 1 ml of extract was diluted to make up to 20 ml with distilled water and slowly shaked in a graduated cyclinder for 15 minutes. 1 one cm layer of foam indicates the presence of saponins.

Detection of Alkaloids (Rosenthaler, 1930; Peach and Trancey, 1955)

0.5 gm of the extract was dissolved in 10 ml of dilute HCL (0.1N) and filtered. The filterate was used to test the presence of alkaloids.

Mayer's test

Mayer's reagent

Readily available from Sd fine chemicals, Mumbai. Filtrate was treated with Meyer's reagent; formation of yellow cream colored precipitate indicates the presence of alkaloids.

Dragendrodroff's test

Dragendroff's reagent

- Dissolve 8 gm of bismuth subnitrate in 20 ml of nitric acid.
- Dissolve 27.2 gm of Potassium iodide in 50 ml of distilled water, mix (a) and (b) and adjust the volume to 100 ml with distilled water.
- Filtrate was treated with Dragendroff's reagent; formation of red colored precipitate indicates the presenc of alkaloids.

Detection of Flavonoids

Alkaline reagent test

To 100 mg 0f extract, few drops of NaOH solution was added in a test tube. Formation of intense yellow color that becomes colorless on addition of few drops of of dilute HCl indicates the presence of Flavonoids.

Detection of Phenolics and Tannins (Kokate, 2001):

100 mg of extract was boiled with 1 ml of distilled water and filtered. The filterate was used for the following test,

a. Ferric chloride test: To 2 ml of filtrate, 2 ml of 1% ferric chloride solution was added in a test tube. Formation of bluish black color indicates the presence of phenolic nucleus.

b. Test for Tannins: To the extract 0.5 ml NaOH was added, formation of precipitate indicates the presence of tannins.

Detection of Phytosterols and Triterpenoids (Paech and Tracey, 1955; Finar, 1959)

0.5 gm of extract was treated with 10 ml chloroform and filtered. The filterate was used to test the presence of Phytosterols and Triterpenoids.

a. Leibermann's test: To 2 ml of filtrate in hot alcohol, few drops of acetic anhydride was added. Formation of brown precipitate indicate the presence of sterols.

b. Leiberman-Bucharat test: To the extract, few drops of acetic acid and concentrated H_2SO_4 were added, deep red ring at the junction of two layers indicates the presence of triterpenes.

Salkowaski test

To the extract solution few drops of Conc Sulphuric acid was added and shaken and allowed to stand, lower layer turns red indicating the presence of sterols.

Detection of fixed oils and fats (Ronsenthaler, 1930)

Oily spot test

One drop of extract was placed on filter paper and solvent was allowed to evaporate. An oily stain on filter paper indicates the presence of fixed oil.

Antioxidant activity test

DPPH free radical scavenging activity

The assay was carried out in a 96 well microtitre plate. To 200 µl of DPPH solution, 10 µl of each of the test sample or the standard solution was added separately in wells of the microtitre plate. The final concentration of the test and standard solutions used were 1000, 500, 250, 125, 62.5, 31.25 and 15.625, 7.812 µg/ml. The plates were incubated at 37^{o} C for 30 min and the absorbance of each solution was measured at 490 nm, using a microplate reader.

Ferric Reducing antioxidant Power Assay

A method developed by Oyaizu, 1986 for reducing power test was used. The above samples were spiked with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was then kept in a 50°C water-bath for 20 min. The resulting solution was then cooled rapidly, spiked with 2.5 mL of 10% trichloroacetic acid, and centrifuged at 3000 rpm for 10 min. The supernatant (5 mL) was then mixed with 5 mL of distilled water and 1 mL of 0.1% ferric chloride. The absorbance at 700 nm was then detected after reaction for 10 min. The higher the absorbance represents the stronger the reducing power.

Lipid peroxidation inhibitory activity

The reaction mixture containing egg lecithin (1ml), ferric chloride (0.02ml), ascorbic acid (0.02ml) and extract or standard (0.1ml) in DMSO at various concentrations was kept for incubation for 1 hour at 37ºC. After incubation 2 ml of 15% TCA and 2ml of 0.37% TBA were added. Then the reaction mixture was boiled for 15 min, cooled, centrifuged and absorbance of the supernatant was measured at 532 nm.

Invitro Cytotoxicity by MTT

Cell lines and Culture medium

HeLa (Human Cervical Carcinoma), HT-29 (Human Colon carcinoma), A549 (Human Lung carcinoma), HaCaT (Human Keratinocyte Carcinoma), VERO (African Green monkey Kidney) cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine

Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in an humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS).

The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Preparation of Test Solutions

For Cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

Procedure

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using DMEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates.

The plates were then incubated at 37° C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37° C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line.

$$\% \text{ Growth Inhibition} = 100 - \left(\frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100 \right)$$

Statistical analysis

Data representative of three independent experiments with similar results were presented as mean ± SD.

RESULTS

Phytochemical Screening

Phytochemical screening of collected plant has been carried out following the methods reported in literature and the results have been reported in Table 1. The whole plant was evaluated for the presence of carbohydrates, glycosides,saponins, alkaloids, flavonoids, phenolics, tannins, phytosterols, triterpenoids, oils, fats.

Table 1. Phytochemical analysis

	TEST	Pet.Ether	Methanol	Chloroform
1.	Carbohydrates	+	+	+
2.	Glycosides	+	+	+
3.	Saponins	+	+	+
4.	Alkaloids			
	a. Mayers Test	-	-	-
	b. Dragendrodrojj's Test	-	-	-
5.	Flavonoids	-	-	-
6.	Phenolics & Tannis			
	a. Ferric Chloride Test	-	+	-
	b. Test for Tannins	-	-	-
7.	Phytosterols & Triterpenoids			
	a. Leiberman – Bucharat	+	+	+
	b. Salkowaski Test	+	+	+
8.	Oils & Fats	-	-	-

Among the extracts, petroleum ether, methanol and chloroform showed the presence of carbohydrates, glycosides, saponins, phytosterols, triterpenoids, saponins showed the presence in petroleum ether and methanol. where as phenolics and tannins showed the presence in methanol extract. Alkoloids, flavonoids, oils and fats are absent in all the extracts.

DPPH radical scavenging activity

The results of DPPH free radical scavenging activity on the three crude extracts are shown in Table 2. The highest % of inhibition was observed at concentration 1000 with an average value of 81.43 (IC_{50} value <62.5 µg/ml) was shown by methanolic extract, chloroform extract showed highest % of inhibition was observed at concentration 1000 with an average value of 95.43, free radical scavenging activity (IC_{50} value 325±0.00 µg/ml),where as petroleum ether showed the moderate activity with % of inhibition at concentration 1000 with an average of 53.44,(IC_{50} value 800±0.00 µg/ml)

Lipid proxidation inhibitory activity

The results of Lipid peroxidation inhibitory activity on the three crude extracts are shown in Table 2. The highest inhibition activity was shown by Petroleum ether extract at highest concentration 1000 with an average value of 73.60 (IC_{50} value 233 ± 3.9), followed by chloroform (IC_{50} value 300 ± 0.0) and methanol (IC_{50} value 650 ± 0.0) with an inhibition at higher concentration 1000 with an average values of 84.54 and 87.74.

Ferric Reducing antioxidant Power Assay

The free radical scavenging power of different extracts increased with increase in concentration of extract. As can be seen in Table 2, there is a clear difference in absorbance in

highest and lowest concentrations. Methanol extract showed stronger reducing power at high concentration, absorbance ranging from 0.524 – 0.272.

Table 2. Antioxidant studies by DPPH

DPPH (Pet-ether)					
Concentration (µg/ml)	% of Inhibition				
	1	2	3	Avg	Std Dev
1000	52.79	53.05	54.50	53.44	0.92
500	45.36	45.62	45.24	45.41	0.20
250	30.50	27.06	29.63	29.06	1.79
125	14.32	18.04	17.20	16.52	1.95
62.5	5.57	6.10	5.29	5.65	0.41
IC50	800	800	800	800.00	0.00

DPPH (Methanol)					
Concentration (µg/ml)	% of Inhibition				
	1	2	3	Avg	Std Dev
1000	82.10	82.76	79.43	81.43	1.76
500	75.28	75.57	73.71	74.86	1.00
250	66.48	68.10	64.00	66.19	2.07
125	61.93	63.79	62.00	62.57	1.06
62.5	51.42	51.15	51.71	51.43	0.28
IC50	<62.5	<62.5	<62.5		

DPPH (Chloroform)					
Concentration (µg/ml)	% of Inhibition				
	1	2	3	Avg	Std Dev
1000	95.48	98.48	92.33	95.43	3.07
500	58.43	58.54	59.51	58.83	0.59
250	46.69	46.65	46.93	46.76	0.15
125	25.90	22.56	28.22	25.56	2.85
62.5	21.08	21.34	20.55	20.99	0.40
IC50	325	325	325	325.00	0.00

Antioxidant studies by FRAP

FRAP (Pet-ether)				
Concentration (µg/ml)	Absorbance			
	1	2	3	Avg
1000	0.171	0.167	0.165	0.168
500	0.108	0.111	0.109	0.109
250	0.061	0.062	0.061	0.061
125	0.044	0.041	0.044	0.043
62.5	0.029	0.031	0.032	0.031

FRAP (Methanol)				
Concentration (µg/ml)	Absorbance			
	1	2	3	Avg
1000	0.52	0.517	0.536	0.524
500	0.502	0.497	0.519	0.506
250	0.445	0.433	0.437	0.438
125	0.396	0.389	0.392	0.392
62.5	0.28	0.269	0.267	0.272

FRAP (Chloroform)				
Concentration (µg/ml)	Absorbance			
	1	2	3	Avg
1000	0.213	0.212	0.218	0.214
500	0.126	0.115	0.116	0.119
250	0.103	0.107	0.113	0.108
125	0.058	0.068	0.068	0.065
62.5	0.045	0.045	0.046	0.045

Antioxidant studies by LPO

LPO (Pet-ether)					
Concentration (µg/ml)	% of Inhibition				
	1	2	3	Avg	Std Dev
1000	72.67	75.47	72.67	73.60	1.62
500	64.60	65.41	65.41	65.14	0.47
250	54.66	54.72	55.28	54.88	0.34
125	18.01	18.87	18.01	18.30	0.49
62.5	14.29	10.06	12.42	12.26	2.12
IC50	230.75	230.75	237.5	233.00	3.90

LPO (Methanol)					
Concentration (µg/ml)	% of Inhibition				
	1	2	3	Avg	Std Dev
1000	85.71	82.61	85.29	84.54	1.68
500	37.14	36.23	35.29	36.22	0.92
250	2.86	5.80	4.41	4.36	1.47
125	0.00	0.00	0.00	0.00	0.00
62.5	0.00	0.00	0.00	0.00	0.00
IC50	650	650	650	650.00	0.00

LPO (Chloroform)					
Concentration (µg/ml)	% of Inhibition				
	1	2	3	Avg	Std Dev
1000	86.69	88.89	87.63	87.74	1.10
500	44.35	40.40	45.36	43.37	2.62
250	34.27	30.30	31.96	32.18	1.99
125	9.07	8.08	6.19	7.78	1.47
62.5	0.00	0.00	0.00	0.00	0.00
IC50	300	300	300	300.00	0.00

IC_{50} values of DPPH, LPO and Absorbance range of FRAP

SAMPLES	IC_{50} VALUES µg/ml		Absorbance Range (1000-62.5)
	DPPH	LPO	FRAP
Pet. Ether	800 ± 0.00	233 ± 3.9	0.581 – 0.331
Methanol	<62.5	650 ± 0.00	0.524 – 0.272
Chloroform	325 ± 0.00	300 ± 0.00	0.514 – 0.245
Standard	Rutin 15.77 ± 0.12	BHA 27 ± 1.00	Quercetin 0.674 – 0.382

Invitro cytotoxicity by MTT

In the present study, cytotoxicity was evaluated on four different cancer cell lines and one normal cell line. All the extracts showed dose dependent activity for the panel of five concentration maintained from 62.5 to 1000µg/ml. All the extracts showed the dose dependent activity. At high concentration (1000µg/ml) extracts showed maximum % inhinbition at concentration. The cytotoxicity of extracts were classified in to three groups according to their CTC50

values - highly toxic (100 – 400), moderately toxic (400 – 800), low toxic (800 – 1000). *W. indica* showed 41% highly toxic, 33% moderately toxic and 26% low toxic. Among the cell lines HeLa cell line showed higher cytotoxicity as the CTC50 values were 103.33 ± 5.77,133.33 ± 5.8 and 623 ± 11.5 µg/ml for chloroform, petroleum ether and methanol extracts respectively. All the extracts exhibited high toxicity towards normal cell line Vero, with CTC50 values ranging from 163±5.8 to 420 ± 10.0µg/ml Overall cytotoxicity values were in the range of 103.33±5.77 to 943.33±11.5µg/ml as shown in Table 3.

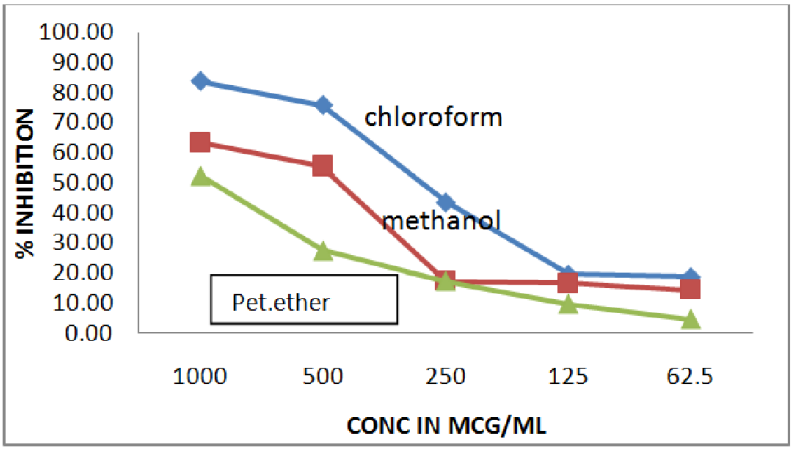
DISCUSSION

The extracts have been evaluated for the qualitative presence of carbohydrates, glycosides, saponins, alkaloids, flavonoids, phenolics, tannins, phytosterols, triterpenoids, oils, fats. Among the extracts, petroleum ether, methanol and chloroform showed the presence of carbohydrates, glycosides, saponins, phytosterols, triterpenoids. Saponins showed the presence in petroleum ether and methanol. where as phenolics and tannins showed the presence in methanol extract.

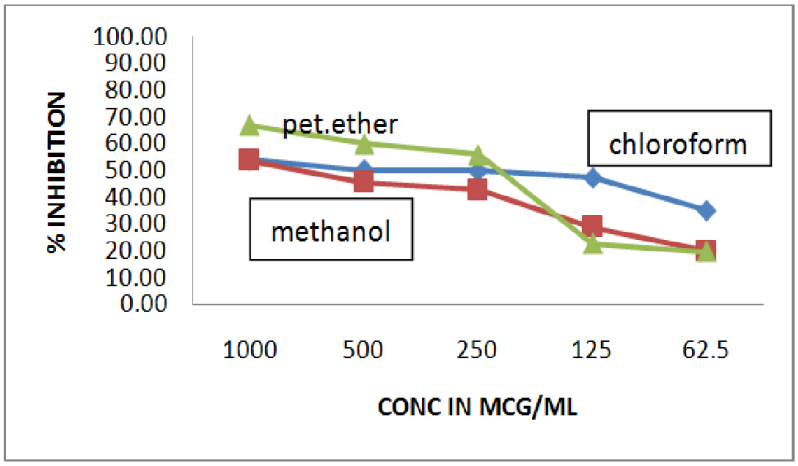
Table 3. Cytotoxicity of extracts against Cancer and Normal cell lines by MTT assay

SL.NO	EXTRACT	CELL LINE (CTC_{50} µg/ml ± SD) Average of 3 replicates				
		HaCaT	A549	HeLa	HT-29	Vero
1.	Pet.ether	226.67±5.8	943.33±11.5	133.33±5.8	612±5.8	420.00±10
2.	Methanol	736.67±5.8	400±10.00	623±11.5	489±5.8	166.67±5.8
3.	Chloroform	623.33±5.8	300±10.00	103.33±5.77	693.33±5.8	163.33±5.8

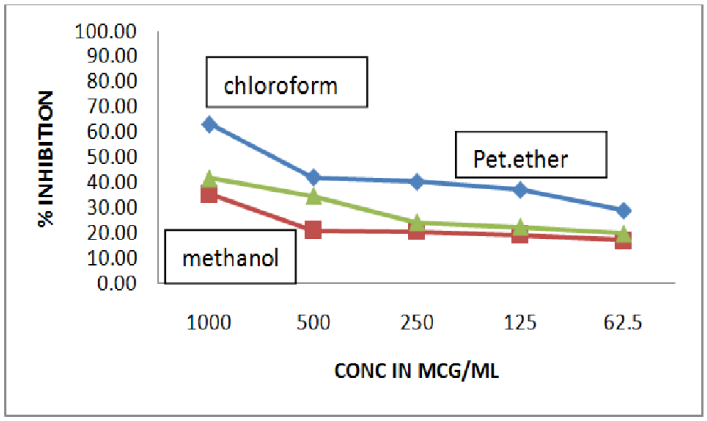
*Average of three independent determinations, 3 replicates, values are mean±SEM.
+CTC50 = concentration of the sample tolerated by 50% of the cultures exposed.



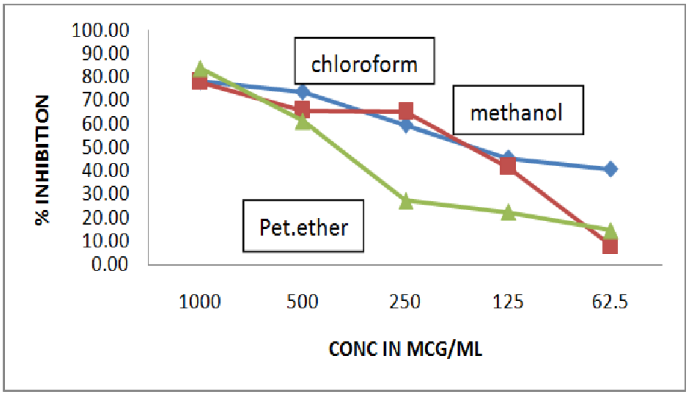
Cytotoxic effect of extracts on HaCaT Cell line



Cytotoxic effect of extracts on HT-29 Cell line

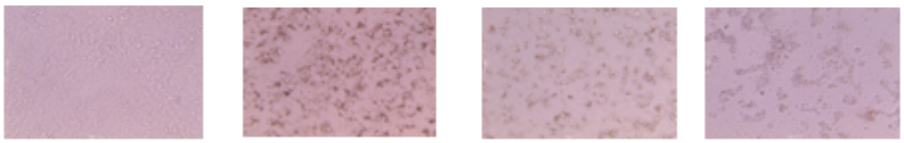


Cytotoxic effect of extracts on Vero Cell line

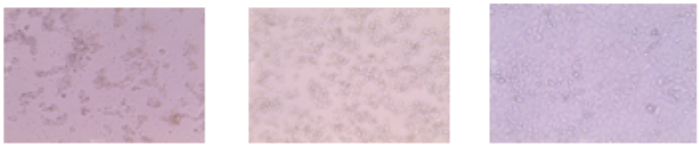


Photos:

A549 Cell line

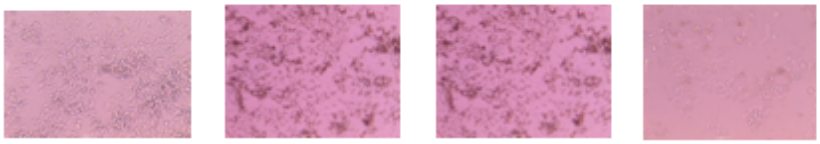


Control Chloroform – 1000 Chloroform- 500 Methanol-1000

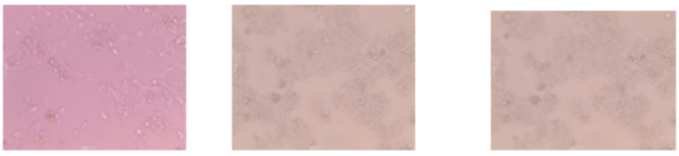


Methanol - 500 Pet. Ether- 1000 Pet. Ether - 500

HT-29 Cell line



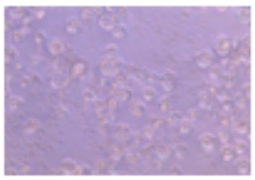
Control Chloroform- 1000 Chloroform- 500 Methanol-1000



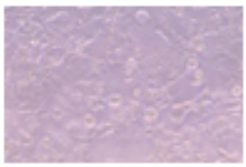
Methanol- 500 Pet. Ether- 1000 Pet.ether – 500

HaCaT Cell line

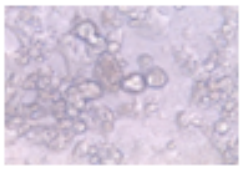
Control



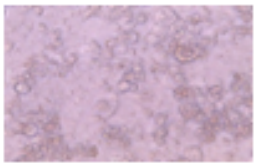
Chloroform-1000



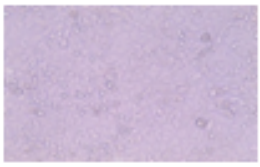
Chloroform - 500



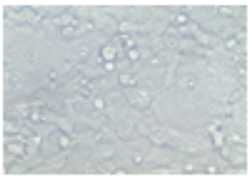
Methanol - 1000



Methanol -500

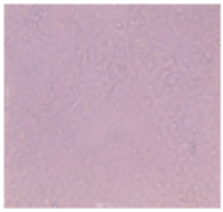


Pet.ether-1000

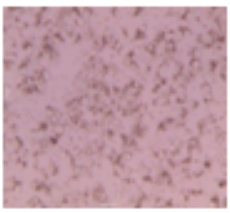


Pet.Ether - 500

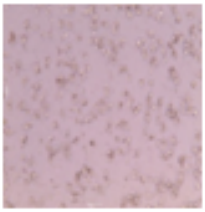
HT-29 Cell line



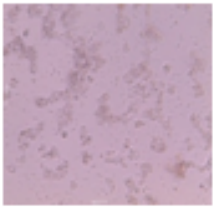
Control



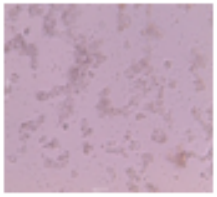
Chloroform – 1000



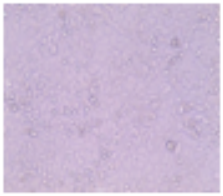
Chloroform - 500



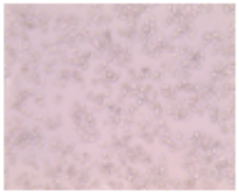
Methanol - 1000



Methanol- 500

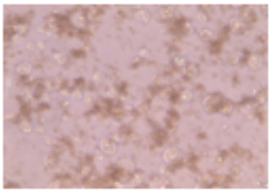


Pet. Ether – 1000

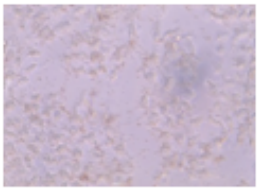


Pet. Ether – 500

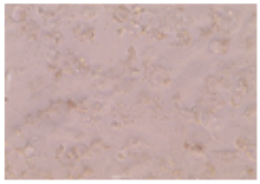
Vero Cell line



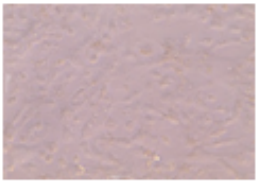
Chloroform - 1000



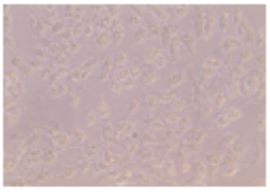
Chloroform- 500



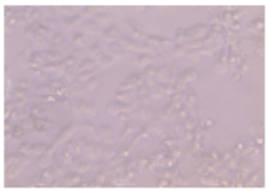
Methanol - 1000



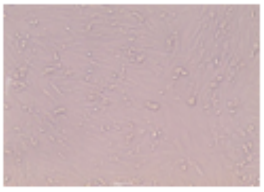
Methanol - 500



Pet.Ether - 1000



Pet Ether- 500



control

Alkoloids, flavonoids, oils and fats are absent in all the extracts. Analyzing the results, it can be observed that the plant contains the largest number of bioactive compounds. The studied bioactive compounds have a broad range of biological activities. For example, phytochemicals such as saponins have anti-inflammatory effects (Ciulei and Istodor, 1995), hemolytic activity, and cholesterol binding properties (Nyarko and Addy, 1990), glycosides are known to lower blood pressure (Ramawat and Dass, 2009) and tannins exhibit antioxidant, antimicrobial and antiviral effects (Ramawat and Dass, 2009). The extracts also revealed to contain triterpenoids, which are known to produce an inhibitory effect on inflammation (Ramawat and Dass, 2009). It is difficult to compare the data with the literature because several variables influence the results. According to some authors, the quantity and the composition of bioactive compounds present in plants are influenced by the genotype, extraction procedure, geographic and climatic conditions, and the growth phase of the plants (Ciulei and Istodor, 1995), (Marinkovic and Vitale, 2008).

Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. The main characteristic of an antioxidant is its ability to trap free radicals. (1, 2). Phenolic and polyphenolic compounds constitute the main class of natural antioxidants present in plants, foods, and beverages and are usually quantified employing Folin's reagent. In this studies antioxidant studies are carried out with most accepted methods such as DPPH, LPO and FRAP. Among the assays carried out DPPH simple method that has been developed to determine the antioxidant activity of foods utilizes the stable 2, 2- diphenyl-1-picrylhydrazyl (DPPH) radical. The structure of DPPH and its reduction by an antioxidant are shown above. The odd electron in the DPPH free radical gives a strong absorption maximum at 490 nm and is purple in color. The color turns from purple to yellow as the molar absorptivity of the DPPH radical at 490 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with a hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization is stoichiometric with respect to number of electrons captured. From the extracts we have taken methanol showed the strong antioxidant activity with IC_{50} value, 62.5, where as the other extracts showed moderate activity with IC_{50} values 325±0.00 and 800±0.00 as shown in Table 2.

Lipid peroxidation assay is based on inhibition of lipid peroxidation. Lipid peroxidation can be initiated by ROS such as hydroxyl radicals by extracting a hydrogen atom from lipids and forming a conjugated lipid radical. This reacts rapidly with oxygen to form a lipid radical until the chain reaction is terminated. The lipid peroxidation adducts may induce the oxidation of biomolecules such as DNA, proteins and other lipids resulting in cellular damage. In our study LPO assay showed the best results in petroleum ether and chloroform extract with IC_{50} values 233±3.9 and 300 ± 0.00.The assay showed the strong % of inhibition at higher concentration such as 1000 µg/ml. FRAP principle is based on higher the absorbance stronger the reducing power. In our study extracts were measured from concentration 62.5 to 1000 µg/ml. Absorbance was measured at 700 nm. The results showed the highest absorbance at highest concentration. Methanol extract showed the maximum absorbance with the range of 0.524 – 0.272 for the concentrations from 62.5 to 1000.

Cytotocity studies were carried by MTT assay. MTT assay is based on principle ability of the cells to survive a toxic insult has been the basis of most Cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The principle involved is the cleavage of tetrazolium salt 3-(4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. The number of cells was found to be proportional to the extent of formazan production by the cells used. Results showed dose dependent activity for the panel of five concentration maintained from 62.5 to 1000µg/ml. All the extracts showed the dose dependent activity.at high concentration 1000µg/ml) extracts showed maximum % inhinbition at concentration. The cytotoxicity of extracts were classified in to three groups according to their CTC50 values - highly toxic (100 – 400) ,moderately toxic (400 – 800), low toxic (800 – 1000).*W. indica* showed 41% highly toxic,33% moderately toxic and 26% low toxic. as shown in Table 3.

Conclusion

The various cell lines employed and *invitro* antioxidant study indicated that *W. indica* is a significant plant that will be useful as an antioxidant and for cytotoxicity. *W. indica* could be a source of new antioxidant and anticancer compounds, this study justified the relevance of this plant as an antioxidants source, but the experiments should be carried out to evaluate the cytotoxicity of plant in *invivo*

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