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PHYTOCHEMICAL CHARACTERIZATION AND ASSESSMENT OF ANTI-CANCER ACTIVITY OF SELECTED MEDICINAL PLANTS AGAINST VERO CELL LINES

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Abstract:

The objective of this study was to discover the scientific source these plants have much inhibitory effects against Vero cell lines. Medicinal plants have secondary metabolites in which more quality against many type of disordered and like flavonoids, phenol, taninns, and some other that have very good and special properties are occur them. DPPH assay screen test on the TLC shows very much anticancer activity. MTT assay proved that these plants are abundant inhibitory activity against VERO cell liens cell. These shows we can reduce that death rate of cancer and also control that cancer that are spared in other body organ and also inhibited the disorder.

Key Words: *TLC, DPPH, MTT, Anti-Cancer, Phytochemicals.*

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INTRODUCTION:

Cancer is type of disease that is spread in any part of body which can cause any infection they are very harmful human body. All benign tumors are not cause problem because that are not spread in other part of body. Undesired weight loss, uncontrolled bleeding, changing in bowel and many other symptoms indicates that body lead to cancer. Tissue, breast, blood or brain cell cancer and many other type of cancer can diagnose in human body that can increase death rate (Newman *et al.*, 2005).

A very strong and good source that have ability to inhibited the cancer and other many type of infection that can cause or diatribe our normally healthy life style also specially they can fight strongly fight against any type of infection other disease that source is plants. In the 1950s, researcher plants are used as an anticancer and found that have very good ability to fight against any type of cancerous cell. The use of natural products has been the single most successful strategy in the discovery of novel medicines". ***Typhonium, flagelliforme***, and ***Murraya, koenigii*** are taken for study and check in lab experiment in which they are found that pant have very good properties that are control that infection or work against very strong side. After some year researcher they work on it and finally proved many type of experiment that plant have many chemical that is topotecan that is more strongly work and inhibited the malignant that can cause lead to cause deathbed (Shoeb *et al.*, 2008).

When fungal bacterial attacks or other types of disease cause in plant that are fight against them and produce many type of chemical they far away from them or kill them. Plants have chemical compounds that fight against fungal disorder because human cancer and fugal cell have same metabolism, when cause cancer in human. Chemotherapy drugs are designed on the basis of toxicity and plant have and plant have many types of chemical that more toxic as like cancer cell when they worked on it the inhibited that.

Human history medicinal plants have been ability to use them. Many type of predators that as insects, fungi and herbivorous mammals that can affected by them, plants have ability to prevents through by chemical they work in and also regulate in their biological function and stops their work that can be create any type of cancer in human cell . When isolated the plant phytochemical they are very less amount that are beneficial for human body cancer cell. these phytochemical work as drugs that are inhibited the progress, and growth of cancerous cell. They are more favorable in pharmacology or pharmaceutical industry and more take advantages, against harmful substances 2008).

Medicinal plant is more important for human history. Researcher is found more than 80%plants have medicine derived from different type of plant parts. Opium, digoxin, quinine, and aspirin to derive from medicine in pharmaceutical to remove remedies that are occur in human body and many other type of infection they may cause can lead to such type of disorder that can lead to cancer.

Modern age of pharmaceutical are very costly as like herbal medicine are very cheap as like that and can easily affordable and easy available for patients. When we do estimate that more than 80% of Asian and Africans are depending on herbal and phytochemical that is derived from plants. Last many decays plants derived medicine use very less amount and now in moder age or during have many research conducts in animal or many scientific proves about plants or herbal medicine and use more amount. The exports level medicinal plants are very much amount increases in global markets.

According to World Health Organization (WHO) more than 80% of the world's community Trust on traditional medicine for their immediately healthcare demand. Importance of herbal medicinal plant in Asia show a long of human communication with the environment. The medicinal rate of plant in some chemical substances that produce a complete philological action on the human body (Hassan *et al.*, 2009). Plants have delivered manhood with herbal medications for numerous diseases for many eras. The plants have very robust power of remedial capability in those crude and phytochemical compounds. Secondary metabolites or some primary metabolite like that show many food processing and have functional effects that are some chemical compound proteins, carbohydrate, fates and used in as a food by animal and human, terpenoids, alkaloids, flavonoids, glycosides and tanins are can take from plants in vast quantity and can regulate the functional effects and a which apply definite functional effects. Those phytochemicals are more frequently accountable for wanted helpful properties. Plants products frequently used to remove the any type of remedy of disorder that presents in the world (Tseng *et al.*, 2006). The death rate of human due to the cancer is increases day by day. We can inhibit the cancer cell through the medicine. By used of medicinal plants and their chemical and parts of plants and vegetable's some herbs can block and inhibited the growth rate of cancer cell in type human and animal body (Abdullaev *et al.*,

2001). Plants have many chemical substance as like secondary compound which some are not directly beneficial for the growth. These compounds have been used as a defense against plant nursing insects and other herbivores. Tumor growth produces due to the disturbance of antioxidant in many tissues of tumor host (Wenger, 2001). Cancer cell can synthesis the hydrogen peroxide that have ability to damage normal tissue and attack other tissue. This show that the direct correlation between in the cancer cell proliferation and change in the antioxidant mechanism. Moreover, some anticancer agent may act as antioxidant (Gupta, 2004).

MATERIALS AND METHODS:

Collection of Samples: *Acacia Senegal willd*, *Acacia chundra* (Rottler) willd, *Albizia lebbeck* (L) Benth, *Albizia Procera* (Roxb), *Parkinsonia aculeate L*, *Cassia fistula L*, *Callistemon citrinus* (Curtis) skeels, *Azadirachta indica*.

Preparation of Sample extracts: The leaves of all plant extracts freshly grained in powered form two-hundred-and-fifty-gram mixed whit 500ml methanol kept at room temperature for 48 h in air tide bottle caped with cover. After 48 h filtered in conical flask with filter paper. All filter material that was kept at hot air oven for methanol evaporated by them. That dry material for more purifying kept in lyopholizer dry freeze that temperature – 46c. at this temperature plant extracts more purify and dry condition (Jonathan *et al.*, 2009).

Qualitative phytochemical analysis: The methanol extracts of these plants were subjected to phytochemical testes for determination of their chemical constituents.

Quantity Phytochemical Analysis:

DPPH Assay:

Preparation of DPPH Salt Solution: DPPH solution was prepared by taking 95ml methanol, 5ml distilled water mixed with 0.4g DPPH salt. 5% DPPH solution was prepared. On the basis of discoloration indicate very good scavenging activity have tested compound (Soler-Evnas *et al.*, 1997).

DPPH Assay by TLC: Tine layer chromatography using for screening of DPPH (Cuendet *et al.*, 1997).That plant extracts are prepared 100mg/ml DMSO after that with the capillary tube lode on that TLC plate with specific tube each plant extract were lode on that for 10 time after completion of 10 time sprayed with 5%DPPH solution that was prepared. After sprayed of DPPH within a second the change color purple back ground into yellow color. The yellow color indicated the presence of antioxidant properties.

Table no.1: DPPH ACTIVITY ON DIFFERENT MEDICINAL PLANTS

S.#	Common name	Botanical Name	Part Used	Family	DPPH
1	Babul	Acacia Senegal willd	Leaf	Fabaceae	+ ve
2	Kath	Acacia chundra (Rottler) willd	Leaf	Fabaceae	+ ve
3	Siris	Albizia lebbeck (L)Benth	Leaf	Fabaceae	+ ve
4	Siris	Albizia Procera (Roxb	Leaf	Mimosaceae	+ ve
5	Jelly bean tree	Parkinsonia aculeate L.	Leaf	Fabaceae	+ ve
6	Amaltas	Cassia fistula L	Leaf	Fabaceae	+ ve
7	Bottlebrush,	Callistemon citrinus (Curtis) skeels	Leaf	Myrtaceae	+ ve
8	Neem	Azadirachta indica	Leaf	Meliaceae	+ ve

DPPH radical scavenging activity: Free radical scavenging activity of methanolic plant extracts was checked by preparing DPPH Salt solution in DMSO and also prepared in methanol. The firstly take plant extract is 1ml in glass tube, add 4ml methanol, mixed with 1ml DPPH salt solution. After no color change but kept in dark place for one night that found color change purple to yellow color that indicated the presence of antioxidant activity. Those found no color change it indicated the not present in antioxidant activity in that plants (Blois *et al.*, 1985).

Total flavonoid determination:

- Methanol (1.5ml)
- Aluminum chloride (0.1ml)
- potassium acetate (0.1ml) (chang *et al.*, 2002).

Determination of tannins:

- ethanolic extract(0.1ml)
- Folin-Denis reagent (0.5ml)
- Sodium carbonate (1ml) (0.5% w/v) (Polshettiwar*et al.*, 2007).

Reducing Power Assay:

- 2.5ml Potassium ferricynaide (10g/l)
- 2.5ml Phosphate buffer (0.2M, pH6.6)
- 2.5ml Trichloroacetic acid (100g/l)
- 0.5ml FeCl3 (1G/L) (Oyaizu *et al.*, 1986).

Total phenols determination:

- Folin ciocalteu(0.5ml)
- sodium carbonate Na2co3 (4ml, 1M) (McDonald *et al.*, 2001).

Nitric oxide scavenging activity:

- Sodium nitroprusside (10Mm,2ml)
- 1ml Griess reagent
- Phosphate buffer 0.5ml (Garrat *et al.*, 1964).

Determination of Super oxide radical scavenging activity:

- NBT (156µM)
- 1ml Nicotinamide adenine dinucleotide (468µM)
- PHOSPHATE BUFFER (100Mm)
- Penazine methosulfate-nicotinamide (60µM) (Ali *et al*,. 2009).

H_2O_2 scavenging activity:

- Alcoholic extracts 30µg/ml
- Phosphate buffer 3.4ml

ABTS radical scavenging assay:

- (7Mm)2,2azinobis-3-ethylbenzothiozoline-6-sulphonic acid
- 2.4mM potassium per sulfate
- 60ml methanol (Ali *et al*,. 2009).

CELL PROLIFERATION ASSAY (MTT):

1. To compare proliferative potential of different groups (3-4,5-dimethylthiazol-2-yl-2,5) diphenyltetrazolium bromide (MTT) assay was performed on the cells cultured on 96 well plates.
2. Monolayer of cells was first washed with phosphate buffer saline (PBS) (Invitrogen Inc., USA).
3. Further it was incubated in 100µl complete medium containing 25µl MTT solution (Invitrogen Inc., USA) (2 - 5mg/ml) for 2 to 4 hrs.
4. MTT is converted into purple colored formazan in living cells which was then solubilized with 10% sodium dodecyl sulphate (SDS) 100 mcl (Invitrogen Inc., USA) and absorbance of solution was taken at 570 nm.(Ruch *et al*,.1989**)**.

RESULTS:

TABLE 2: Phytochemical and Antioxidative Characterization (Total Phenolic, Tannin and Flavonoid Contents in The Plant Extracts (Methanol).

Plant name	DMSO 415nm	Tenins 755nm	Phenols 765nm	Flavonoide 546nm	ABST 743nm	H_2O_2 230nm	RPA 700nm	SOD 560nm	NO 546nm	DPPH
Acacia Senegal willd	3.00	0.125	3.96	0.910	1.888	2.128	1.644	0.06	0.091	+ve
Acacia chundra (Rottler) willd	3.00	0.084	0.192	0.052	0.945	2.079	1.906	0.071	0.052	+ve
Albizia lebbeck (L)Benth	3.00	0.089	0.190	0.071	2.771	2.049	2.079	0.061	0.071	+ve
Albizia Procera (Roxb)	3.00	0.11	0.427	0.105	0.917	2.331	2.189	0.099	0.105	+ve
Parkinsonia aculeate L.	3.00	0.083	1.267	0.057	1.686	2.045	2.804	0.064	0.057	+ve
Cassia fistula L.	3.00	0.084	0.135	0.071	0.453	2.022	1.684	0.055	0.071	+ve
Callistemon citrinus (Curtis) skeels.	1.227	0.07	0.209	0.209	2.471	1.274	2.797	0.062	0.209	+ve
Azadirachta indica.	1.254	0.09	2.011	0.209	2.638	1.266	1.771	0.061	0.209	+ve

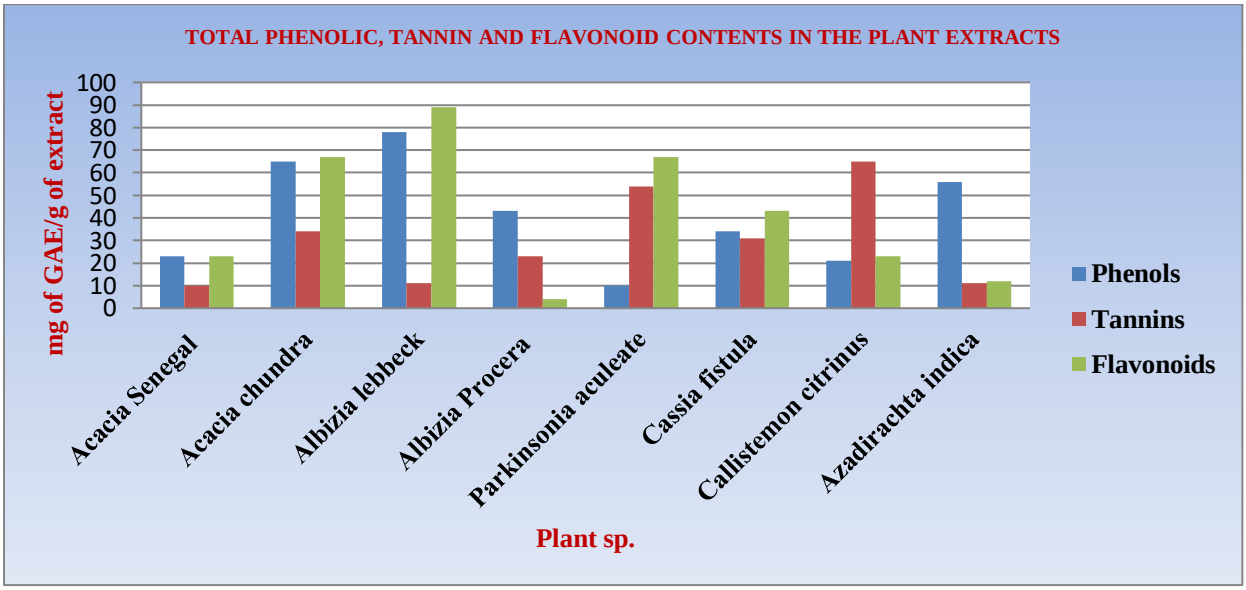

FIGURE 1: Phytochemical and Anti-oxidative Characterization in the Plant Extracts (Methanol) Phytochemical Analysis

The screening of secondary metabolites performed by using color and chemical reagent on the dried leaves of methanol plant extracts that are mention in material and method. The secondary metabolites are qualitatively performed by DPPH assay sprayed on TLC plate. Phenols, flavonoids, tannins, reducing assay, nitric oxide, super radical, ABTS radical scavenging, hydrogen peroxide test was performed of that plant extracts of these species. Flavonoids, tannins, phenols, these have very worthy oxidative properties or good strength against fight any type of disoreder. Phenols and poly phenol have been reported as a medicnal activity and also potent for physiological activity. That present in flavonoids that contain hydroxyl group due to the presences of are more accountable for the radical scavenig activity in that plant showed in figure.2.

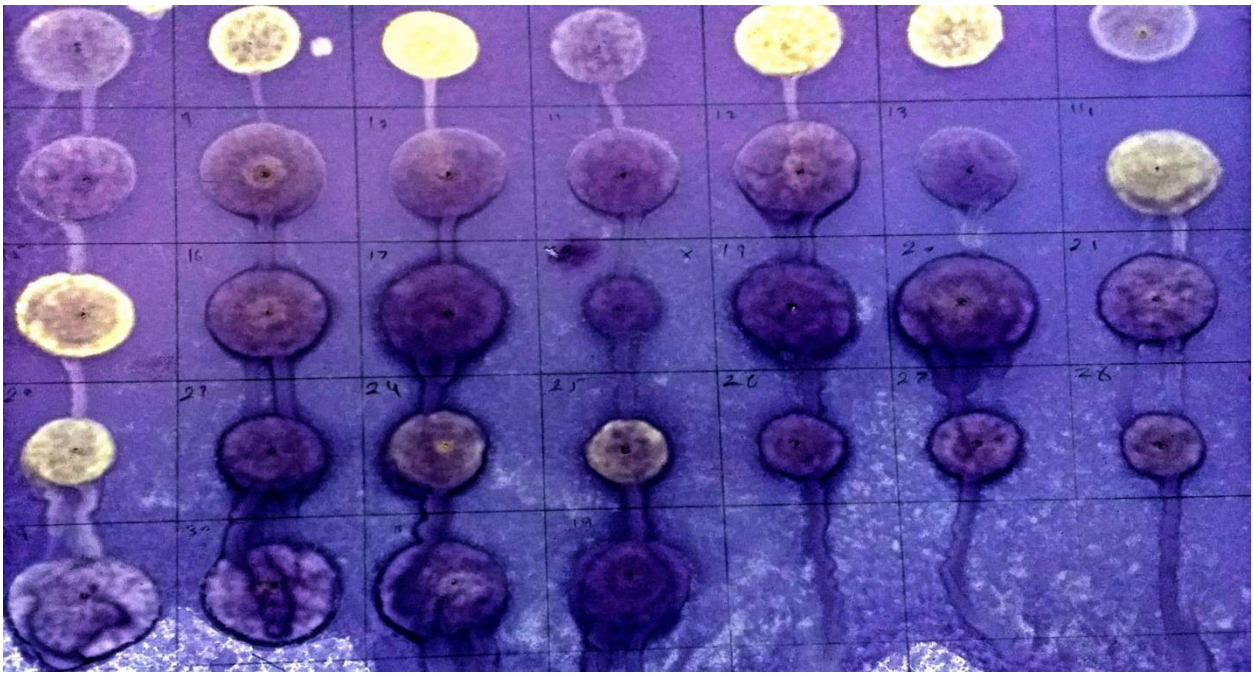

FIGURE 2: TLC Method for DPPH radical scavenging activity

DPPH radical scavenging activity: Present study showed that all plant extracts have found more effective scavengers against DPPH radical. Methanol plant extracts showed the highest DPPH radical scavenging ability. That showed color change with in a second purple to yellow. That method occurs when donates one hydrogen of ion due to color change are occur . Plant extracts decrease the color of DPPH due the occurrences of the power of hydrogen they contributing ability. DPPH anion scavenging power of extracts. The change in color purple DPPH to yellow that visibly showed and they give verified the effect of extracts as an antioxidant showed in figure.2.

Reducing Power Assay (RPA): The possible of antioxidant compounds may help as more important pointer that antioxidant activity. The reducing power assay has been performed when plants extracts have capability that have antioxidant and can be evaluated of Fe^{+} and that have showed reducing power high on the basis of the plant extract concentration that are increased within the increasing of plant extracts concentration. The extracts showed ability due to the presences of ferric reducing power that are very potent.

Nitric Oxide Activity: The alcoholic plant extracts of leaves of all plants showed effective and have induced of free radical that are present in which the concentration of plants extracts. The plant extracts exhibited inhibition activity against alcoholic extracts. That inhibition due to the nitric oxide is free carcinomas that are reacts oxygen to in the form of nitrite that generate the oxygen to react with sodium nitroprusside. That all reaction process oxygen compete the nitric oxide ant inhibited the nitrite formation. Showed in table. 2.

Superoxide Assay: The reactive oxygen species is a destructive the biological system**.** The alcoholic extracts showed effective superoxide radical scavenging activity. That result recommended superoxide radical scavenger has very low efficiency when compared with standard show in table.2.

ABTs Radical Scavenging Activity: The effectiveness of ABTs radical scavenging activity process that was showed for plant extracts. The plant extracts have ability more in alcoholic extracts that concentration 100µg/ml. That assay in which hydrogen peroxide no work itself they work cytotoxicity occur the give high hydroxyl radical in the cell show in table.2.

MTT Assay: Cassis fistula and Acacia Senegal shows the maximum % of cytotoxicity followed by Acacia Chundra, Albizia Lebbeck, Callistermon citrinus and Azadirachta indica respectively. The minimum % of cytotoxicity expressed by Albizia Procera and Parkinsonia aculeate show in figure 3.

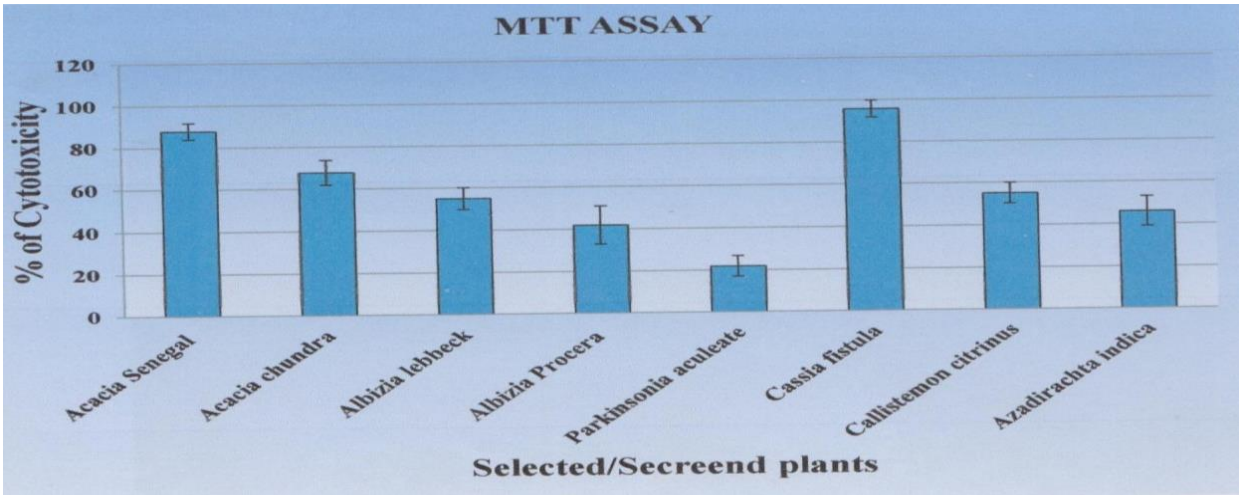

FIGURE 3. MTT ASSAY CONTENTS

DISCUSSION:

Present study reveals that plants have very good inhibitory effect against the cell toxicity i.e. ***Acacia Senegal, Acacia Chundra, Albizia Lebbeck, Callistermon citrinus, Azadirachta indica, Albizia Procera and Parkinsonia aculeate***. Plants have very good inhibitory activity as methanolic plant extracts against VERO cell lines that causes many types of cell toxicity. The methanolic extracts have antioxidant activity and inhibited the ROS actions. All selected plant have good anticancer activity to due to phytochemical that are presents in those plants. That test performed on the basis of in which process hydrogen bond donating antioxidant due to the formation of DPPH in mathanolic extracts in which less similar anion scavenging power. The color change of plants extracts violet into yellow clearly by the effect of cancerous and any toxic substances that are very harmful and can break the biological systems. The reducing power assay capability of a compound may as an indicator of its potential antioxidant activity it evaluated by the transformations of Fe^{2+} in the presence of the extracts in the oxidant activity are present in that plants. The athanolic plants extracts in which nitric oxide activity the induced the activity in which that are present in that are inhibited the alcoholic plant extract but in which that are ascorbic acid showed inhibition activity is less then alcoholic plants extracts the alcoholic plants extracts fight against many cancerous disorder that are present in human body. The inhibited nitric formations in which whenever convert not in nitrate in which that proved the plants extracts have very good antioxidant activity. The SO are very toxic for human and that also damage the cellular components in the biological system. The presence of phytochemical components they inhibited the quenching activity. They showed that plants extracts have very less efficiency as compared to standard. The test performed ABTS radical scavenging process was in which they affect as very good inhibition activity. These are shows in graph and in the table in which that are present in which process that is present in which. All phytochemical that are present specially secondary metabolites that are in plant and that provide immunity against production of any type of carcinoma and any type of inflammatory disorder that are present in any type of cell growth in that is inhibited them and those showed very positive effective against. These plants extracts have very good anticancer activity against Vero cell line of selected plants.

Cassis fistula and ***Acacia Senegal:*** shows the maximum % of cytotoxicity followed by *Acacia Chundra, Albizia Lebbeck, Callistermon citrinus and Azadirachta indica* respectively. The minimum % of cytotoxicity expressed by *Albizia Procera* and *Parkinsonia aculeate* are very good effect against

VERO cell lines and they inhibited the any type of infection that are occur in cell.

CONCLUSION:

Herbal plants have the ability to combat against infection. The extracts of the medicinal plants that were taken from many areas of the world. Anticancer activity of medicinal plant were observed by implementation experiment. Medicinal plant are used for the cure of the numerous ailment due to the capability to work against the infection and man synthetic drug herbal plants are free from many side effect that are exhibited in case of the synthetic drugs. These plants having become the points of focus because of the advantage they are having. Modern drug are designed by the help of the herbal plants. These herbal plants are used as source of medicine because they are naturally occurred and the potential to act against various infection and disease.

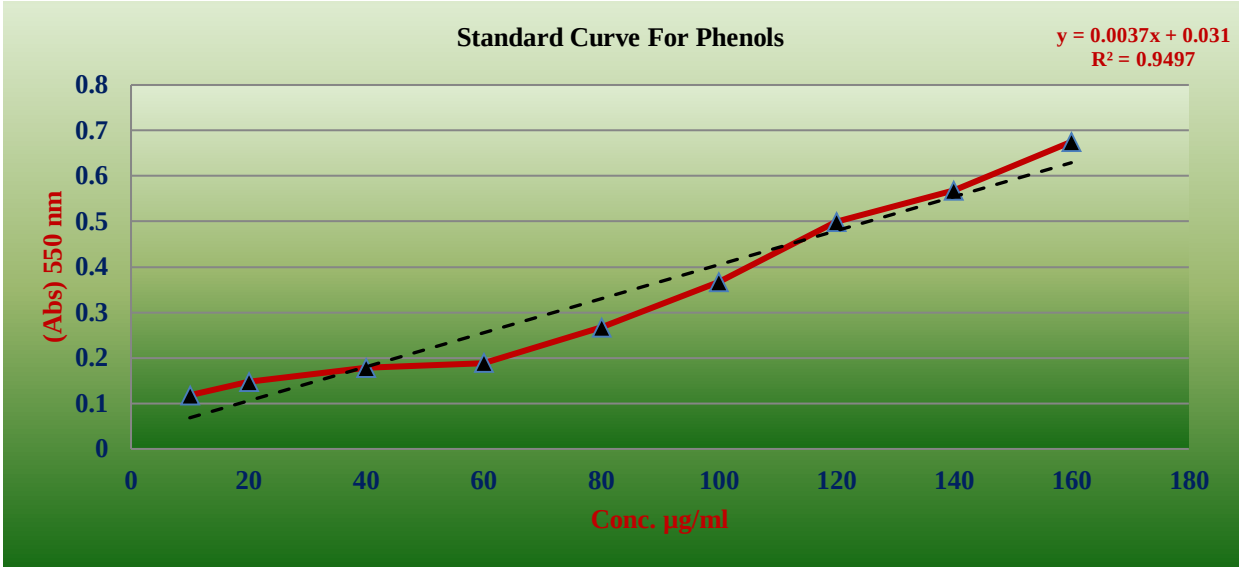

FIGURE 3: STANDARD CURVE FOR PHENOLIC CONTENTS

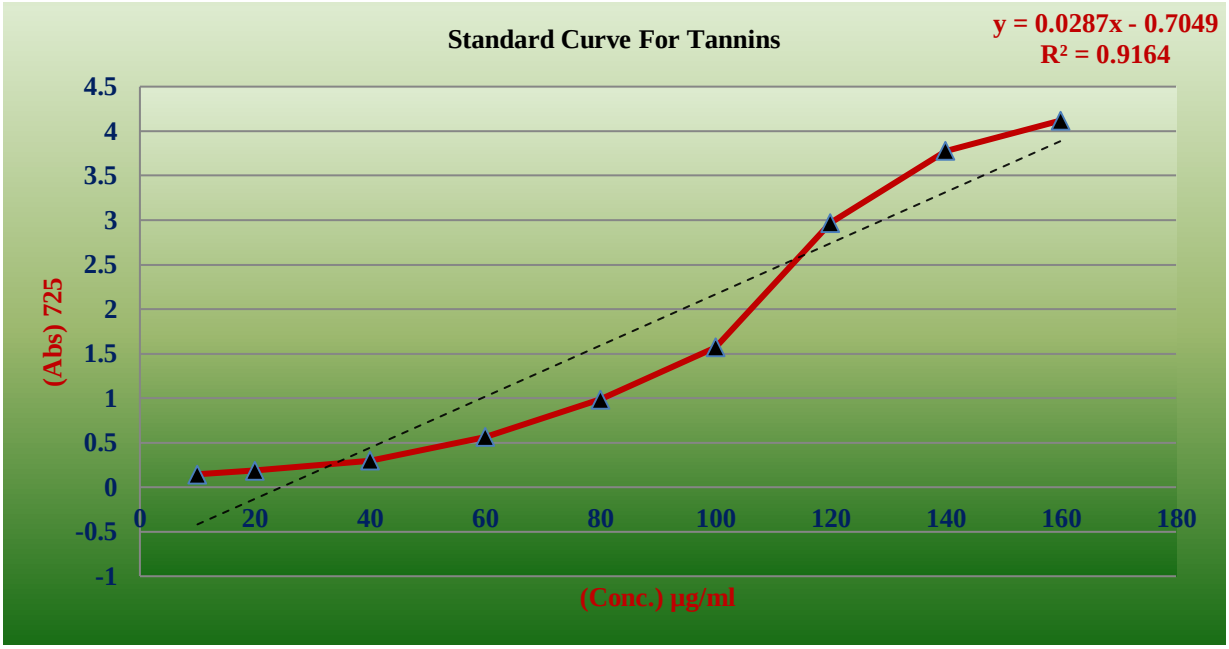

FIGURE: 4 STANDARD CURVES FOR TANNIN CONTENTS

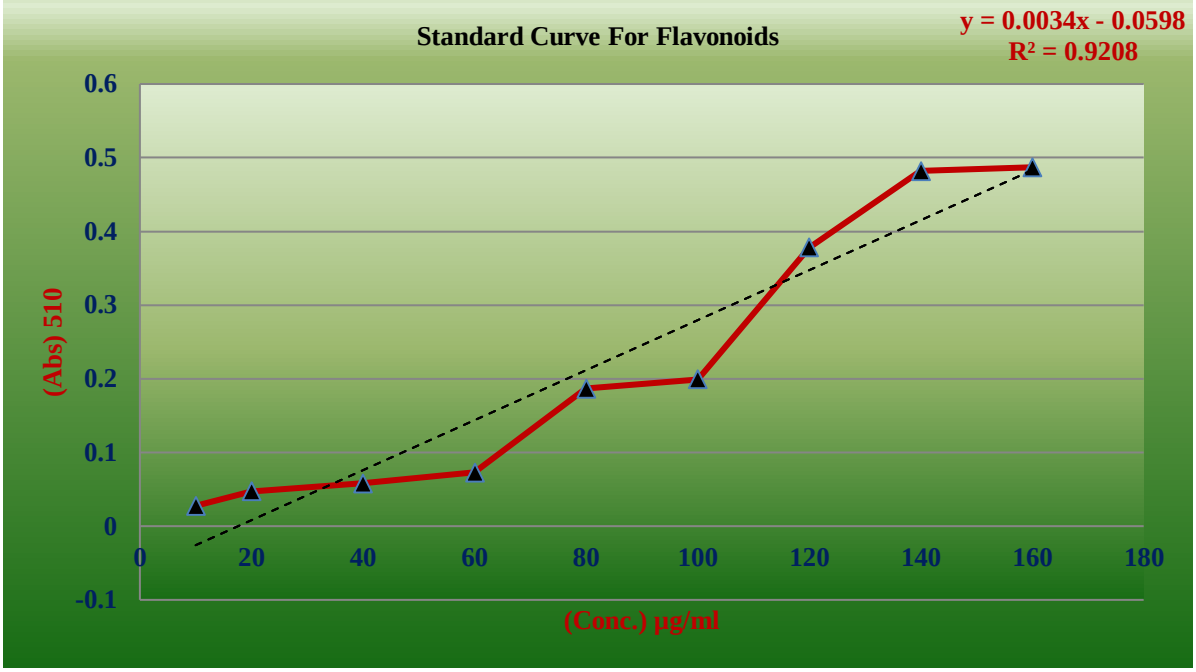

FIGURE: 5 STANDARD CURVES FOR FLAVONOIDS CONTENTS

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