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PHYTOCHEMICALS, ANTIBACTERIAL, ANTIFUNGAL AND CYTOTOXICITY OF SOME SELECTED MEDICINAL PLANTS FROM BHANDARDARA AREA, MAHARASHTRA

Kakad S. L.[1] and Dhembare A. J.[2]

[1]*Department of Biotechnology,*

[2] *Zoology, P. V. P. College, Pravaranagar-413713, Ahmednagar, MS, India*

Abstract

This attempt was assigned to evaluate the phytochemical constituents, antifungal, antibacterial and cytotoxicity of eleven medicinal plants from Bhandhara area. The phytochemicals such as steroids, anthraquinone, tannins, saponin, alkaloids, phlobatannin, glycosides, quinine and flavonoids were tested qualitatively. Antifungal, antibacterial and cytotoxicity was evaluated with the respective strains in laboratory condition. The total contents of flavonoids were examined quantitatively in eleven plant species and showed maximum (23%) contents from Terminalia chebula (Retz) followed by Vitex nigundo (L). The antibacterial, antifungal and cytotoxicity were conducted. The highest antibacterial potential was revealed from the extracts of Termanalia chebula (Retz) against E. coli, followed by Glycyrrhiza glabra (L) to Pseudomonas pudita. Antifungal activity of eleven plants was tested against the four fungal strains and showed that highest potentials in Termanalia chebula (Retz) while followed by Vitex nigundo (L) and Bacopa mannieri (L). The cytotoxicity was reported on chick embryo cell line against eleven species for their establishment and two species were showed high percent viability fromTerminalia chebula (Retz) (72.2%) followed by Acacia catechu (L.) (65.8%). The viability varies species to species. The phytochemicals are the indicatives of the plant species could be a possible source of obtaining new and effective source of herbal medicine.The antibacterial, antifungal potential activities are better source for further herbal medicine.

Keywords: Phytochemicals, antibacterial, antifungal, cytotoxicity, plant extract.

I. INTRODUCTION

Various natural products have been isolated from number of plant species. These isolated natural products have remarkable variety of compounds having unusual structures, many of which have found uses in the cosmetic dye and pharmaceutical industries. In addition these compounds are plant growth regulators, fungicides, insecticides, pest control agents and repellents of herbivores. With increase in awareness about environment and sustainable development natural products found to be new area of research due to its biodegradable nature and production from renewable resources.

Nature has provided a store house of remedies to cure all ailment of mankind. Use of plants as a source of medicine has been inherited from the onset of human civilization and is an important component of the healthcare system. In recent years, chemical and biological assays have begun to play an important role in ethnobotanical studies.Medicinal herb is considered to be a chemical factory as it contains multitude of chemical compounds [1]. Today is growing interest in chemical composition of plant based medicines. Several bioactive constituents have been isolated and studied for pharmacological activity.

Plants derived compounds are playing an important role in the development of several clinically useful medicines [2]. Workers first started extracting and isolating chemicals from plants in the 18th century and since the time we have grown a custom of looking at herbs and their effects in terms of the active constituents they contain. It is generally accepted that plants based medicines are better than synthetic drugs which are much safer for human beings.

Phytochemicals are secondary metabolites of plants and having protective properties. These metabolites are showed structural similarities to those of intermediately molecules of animals. These molecules can interact in a similar mode and responsible for the desirable properties [3]. These molecules have been considered as nutritional importance in the diseases prevention [4]. These molecules are also impotent in the pharmacological actions. Most phytochemicals have antioxidant, anticancer, antibacterial, antifungal, antihelmintic, etc, activities and protect cells from damage and diseases [5, 6]. However, the finding of single molecule having both phytochemical and pharmacological properties would be great therapeutic importance. Other workers from the local area were tested some medicinal plant species for phytochemical, antibacterial, antifungal and cytotoxicity activities [7, 8, 9, 10]. Out of these species another species were assigned to evaluate phytochemical, antibacterial, antifungal and cytotoxicity properties from the localmedicinal plant species.

II. MATERIALS AND METHODS

Chemicals: All chemicals and reagents used in this study were of analytical grade and obtained from Merck Company, Germany.

Plant: Eleven plant species were collected from the local area of Bhandardara. Further, plant species were identified and registered by Herbarium, Department of Botany, P. V. P. College, Pravaranagar, and Ahmednagar, MS, India.

Preparation of extracts: The plant leaf material was dried under shade at room temperature for about 10 days. The dried plant samples were powdered by mechanical grinder and sieved to give particle size 50 to 150 mm. The powder was stored in polythene bags at room temperature before extraction. Powder (25 g) was filled in the thimble and extracted successively with 70% methanol (methanol: water; 70: 30) and ethyl acetate: chloroform: ethyl alcohol (40: 30: 30) solvents in soxhlet extractor for 48 hours. The extracts were concentrated to dryness using rotary evaporator and crude extracts were tested.

Table 1: Qualitative screening of phytochemicals in plants species.

Sr. No.	Plants	Steroids	Anthraquinone	Tannins	Saponin	Alkaloids	Phlobatannin	Glycosides	Quinone	Flavonoids
1	*Acacia catechu* (L.)	+	+	++	+++	++	++	-	+++	-
2	*Bacopa mannieri* (L.)	+	-	-	+++	+++	-	-	++	++
3	*Chlorophytum borivilianum* (L.)	+	-	+	+	++	-	-	+++	-
4	*Cinnamomum verum* (L.)	+	+	++	++	++	++	+	+++	-
5	*Cyclea peltata* (Lam)	+	-	++	++	++	-	-	+++	+
6	*Dioscora bulbifera* (L.)	+	+	-	+++	++	-	++	+	-
7	*Fagonia arabica* (L.)	+	-	+	+++	++	-	-	++	+
8	*Glycyrrhiza glabra* (L.)	+	-	-	++++	++++	++	+	+	++

9	*Inula racemosa* (Hook f)	+	-	++	+	++	+	-	+++	+
10	*Terminalia chebula* (Retz)	++	-	+++	++	++	-	-	++	+++
11	*Vitex nigundo* (L.)	+	+	+++	++	+++	++	-	+++	++

Qualitative: + = low, ++ = moderate, +++ = high, +++ = higher and - = not reported

Table 2: Total flavonoids in plants species.

Sr. No.	Plant species	Total flavonoids (%)
1	*Acacia catechu* (L.)	1.2
2	*Bacopa mannieri* (L.)	1.6
3	*Chlorophytum borivilianum* (L.)	2.3
4	*Cinnamomum verum* (L.)	1.9
5	*Cyclea peltata* (Lam)	2.6
6	*Dioscora bulbifera* (L.)	3.2
7	*Fagonia arabica* (L.)	3.8
8	*Glycyrrhiza glabra* (L.)	4.7
9	*Inula racemosa* (Hook f)	2.1
10	*Terminalia chebula* (Retz)	23.0
11	*Vitex nigundo* (L.)	7.8

Phytochemical analysis: In recent years, secondary plant metabolites (phytochemicals) have been extensively investigated as a source of medicinal agents. Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of various infections. These are the qualitative tests performed to analyze the presence or absence of various phytochemicals such as alkaloids, tannins, flavonoids etc. in plant extract [11].

Total flavonoids: The quantative analysis of total flavonoids content was determined using aluminium chloride colorimetric method with slight modifications [12]. Dried extracts (10 mg) was dissolved in 10 ml of 70% methanol. Extract solution (1000 µl) was mixed with 1 ml of 2% aluminium chloride and 6 ml of 5% potassium acetate. Then the mixtures were allowed to stand for incubation at room temperature for 40 minutes and the absorbance of the reaction mixture was measured at 415 nm using spectrophotometer. Total flavonoids were expressed in mg of quercetin equivalent per gram of the dry plant extract (mg QE/g).

Antibacterial test: The antibacterial activity was carried on bacterium such as *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas putida*, *Pseudomonas aeroginosa*, *Salmonella typhimurium* and *Klebshiella pneumonia.* It was carried on aqueous extracts and well diffusion methodfor solvent extracts. The molten Muller Agar (HiMedia) was inoculated with the 100 µl of inoculum (1x106 CFU) and poured in to sterilized petri plate. For agar disc diffusion method, the disc (0.7 cm) was saturated with 1000µl of test compound, allowed to dry and was introduced on the upper layer of the seeded agar plate. The plates were incubated over night at 37^0C. Microbial growth was determined by measuring diameter of the zone of inhibition. For each bacterial strain control was maintained in pure solvent were used instead of extract. The result was obtained by measuring the zone of inhibitions in a diameter. The experiment was repeated three time and mean values were used (Table 3). The obtained data were compared with the standard antibiotics penicillium (100µl/disc) and gentamicin (10µl/disc).

Table 3: Antibacterial activities of plant species.

Sr. No.	Plant sample	Bacterial strains (zones of inhibition in mm diameter)					
		Gram positive bacteria			*Gram negative bacteria*		
		Bacillus subtilis	*Escherichia coli*	*Pseudomonas putida*	*Pseudomonas aeroginosa*	*Salmonella typhimurium*	*Klebshiella pneumonia*
1	*Acacia catechu* (L.)	2	2	--	1	--	1
2	*Bacopa mannieri* (L.)	1	2	2	--	--	2
3	*Chlorophytum borivilianum* (L.)	--	2	1	--	--	1
4	*Cinnamomum verum* (L.)	2	-	3	1	--	-
5	*Cyclea peltata* (Lam)	--	2	1	--	2	2
6	*Dioscora bulbifera* (L.)	2	1	3	1	1	--
7	*Fagonia arabica* (L.)	4	--	--	4	1	1
8	*Glycyrrhiza glabra* (L.)	5	--	7	1	3	--
9	*Inula racemosa* (Hook f)	--	2	1	--	2	--
10	*Terminalia chebula* (Retz)	3	9	--	2	4	2
11	*Vitex nigundo* (L.)	4	3	2	--	2	--

Antifungal test: The antifungal activity was carried on fungusstrains such as *Aspergillus niger, Trichoderma viridae, Fusarium oxysporum* and *Alternaria solani.* The activities were carried out on aqueous extracts; a modified micro dilution technique was used. The fungal spores were washed from the surface agar plate with sterilized 0.85% saline containing 0.1% Tween 80 (v/v). The spore suspension was adjusted with saline to a concentration of approximately 1.0- 107 in final volume of 100 µl per well. An inoculate were stored at 4^0C for further use. Dilution of inocula was cultured on solid potato dextrose agar to verify the absence of contamination and to check the validity of inoculums. The diameter of the inhibition zone was measured in mm (Table 4).

Table 4: Antifungal activities of plant species.

Sr. No.	Plant sample	Fungal strains (zones of inhibition in mm diameter)			
		Aspergillus niger	*Trichoderma viridae*	*Fusariumoxysporum*	*Alternariasolani*
1	*Acacia catechu* (L.)	2	1	--	1
2	*Bacopa mannieri* (L.)	6	--	2	4
3	*Chlorophytum borivilianum* (L.)	2	--	3	2
4	*Cinnamomum verum* (L.)	3	2	--	1
5	*Cyclea peltata* (Lam)	3	1	2	--
6	*Dioscora bulbifera* (L.)	--	1	--	2
7	*Fagonia arabica* (L.)	3	--	3	2
8	*Glycyrrhiza glabra* (L.)	2	1	--	--

9	*Inula racemosa* (Hook f)	3	--	3	2
10	*Terminalia chebula* (Retz)	9	7	--	2
11	*Vitex nigundo* (L.)	5	2	5	--

Cytotoxicity test: Fibroblast cell line was established from chick embryo using DMEM medium supplemented with serum (Foetal Bovine Serum 10% and Gentamicin 50 µg/ml). A fibroblast cells line (5 ml) suspension was added to six well microtitre plates. Different concentrations (50 µl, 100 µl, 150 µl) of leaf extracts were added to each well in triplicates. The microtitre was incubated aseptically in CO_2 incubator for 24 hours at 37^0C. After incubation cells were disaggregated using trypsin (0.25%). Percent viability was made using Trypan Blue on Neubaure Chamber and percent viability was calculated (Table 5).

Table 5: Viability on chick embryo fibroblast cell line.

Sr. No.	Plant species	% viability
1	*Acacia catechu* (L.)	65.8
2	*Bacopa mannieri* (L.)	50.4
3	*Chlorophytum borivilianum* (L.)	59.9
4	*Cinnamomum verum* (L)	35.3
5	*Cyclea peltata* (Lam)	41.6
6	*Dioscora bulbifera* (L.)	36.9
7	*Fagonia arabica* (L.)	28.8
8	*Glycyrrhiza glabra* (L.)	29.5
9	*Inula racemosa* (Hook f)	21.7
10	*Terminalia chebula* (Retz)	72.2
11	*Vitex nigundo* (L.)	27.5
12	Methanol control	51.4

III. RESULTS AND DISCUSSION

The result of phytochemical analysis of selected eleven plant species extract showed presence of alkaloids, anthraquinone, glycosidase, flavonoids, phytobatannin, saponins, steroids, tannins, and quinine (Table 1). These phytochemical vary species to species was reported as low, moderate, high and highest qualitative levels. The quantative screening revealed that highest level in saponin, alkaloids and quinine and low in the anthraquinone and glycosides.

These are natural products from local medicinal plants, either as pure compounds provide opportunities for new drug. Due to an increasing demand for chemical diversity in screening programs, seeking therapeutic drugs from natural products, interest particularly in edible plants has grown throughout the world. Botanicals and herbal preparations for medicinal usage contain various types of bioactive compounds. Plant secondary metabolites such as alkaloids, phenols, tannins, glycosides, terpenoids, saponins, flavonoids and steroids had been implicated in their ability to inhibit the formation of pro-inflammatory signalling molecules such as prostaglandin or leukotrienes [13].

Alkaloids: The alkaloids phytochemical contents reported in all the screened plant species. It was ranged from moderate to higher qualitative level. However, it was higher in the *Glycyrrhiza glabra*

(L) species and moderate in all species. The alkaloid and flavonoid content of plant materials has severally been reported to be a major antioxidant, anti-inflammatory and analgesic active principle [14].

These are basic natural contents present in plants. They are as one or more heterocyclic nitrogen atoms and are found in the form of salts with organic acids. Alkaloids are the efficient therapeutically significant plant substances. Pure alkaloids and their synthetic derivatives are used as basic medicinal agents because of their analgesic, antispasmodic and antibacterial properties. The high alkaloid contents in the plants are presently used in the therapeutic.

Anthraquinone: It was revealed from table 2 that out of eleven species screened only four species reported anthraquinone contents in low level while seven species not reported.

Anthraquinone is one of the compound occur naturally in plants, fungi, lichens and insects. They serve as a basic skeleton for their pigments. Natural anthraquinone is studied due to its wide range of applications. Anthraquinone is used as laxatives mainly from their glycosidic derivatives and also used in the treatment of fungal skin diseases [15]. Anthraquinone and its derivatives are found in slimming agents and important for their cathartic and presumed detoxifying action which may cause nausea, vomiting, abdominal cramps and diarrhea [15]. Anthraquinone had shown antioxidant property such as BHA (96%), anthrone (95%), alizarin (93%), aloe-emodin (78%), rhein (71%), emodin (36%) and anthraquinone (8%) [16]. The natural and synthetic anthraquinone have widespread applications throughout industry and medicine [17]. Plant containing anthraquinone are being used for cosmetics, food, dye and pharmaceuticals due to their wide therapeutic and pharmacological properties [18].

Glycosidase: It was revealed from the screened data that the three species reported glycosidase in low level while other all species not reported glycosidase contents.

Gglycoside's are compounds which yield glucose, hydrogen cyanide and aldehyde or ketone upon hydrolysis with an acid or enzyme. Dietary exposure to cyanide occurs mainly in the consumption of food stuffs rich in endogeneous cyanide in the form of cyanogenicglucosides. It could be lethal as it intercalates with cytochrome oxidase for aerobic function [19]. Only free cyanide (CN-) is toxic and if hydrolysis does not occur, the glycoside remains stable and the food using this product becomes safe.

Flavonoids: This plant phytochemical was reported from seven plant species screened and showed higher in *Terminalia chebula* (Retz) while for plant species showed nil report.

Theplant flavonoids have biological functions such as protection against allergies, inflammation, free radical, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumor [20, 21]. Flavonoids are water soluble antioxidants and free radical scavengers which prevent oxidative cell damage, having strong anticancer activity which protect against the carcinogenesis [21]. Flavonoids lower the risk of heart diseases. The antioxidant potentials of plants have been linked with the flavonoids contents. In addition, the therapeutic potentials of plants have been linked with their antioxidant potentials [22, 23]. The flavonoids from the plants may provide anti-inflammatory activity. Thus the high alkaloid and flavonoid contents of the plants explain their therapeutic use in herbal medicine especially in the treatment of wounds, burns and ulcers. The anti-inflammatory property of flavonoids is believed to result from inhibition of cyclo-oxygenase enzyme [24]. The flavonoids extract was effective in the treatment of capillary fragility, retinal haemorrhage in hypertension, diabetic retinopathy, purpura, rheumatic arthritis, radiation disease, habitual abortion, frostbite, anaphylactic shock, experimental cancer and in the prevention of chromodacroyorrhoea produced by dietary and environmental stress [25].

Phlobatannin: It was observed in the present task that five plant species revealed low level of phlobatannin content while six plant species reported nil report out of eleven plants screened.

The phlobatannin components of kino gum and Butea gum have been purified and rendered free from water and ether soluble impurities. By methylation and acetylation almost colourless methyl ethers and acetates have been obtained. They exhibit marked optical activity thus supporting the idea that the

phlobatannin are probably hydroxyflavan derivatives. A comparison of the properties of the phlobatannins from the two sources and of their derivatives indicates that they are identical.

Saponin: It was showed from the data that saponin varies species to species. It was higher in *Glycyrrhiza glara* while low in the *Inula racemosa* (Hook F) and *Chlorophytum borivilianum* (L).

Saponins are glycosides of triterpenes, steroids and alkaloids, which occur in plant but notexclusively. The saponins are steroidal, triterpenoidal or alkaloidal depending on the nature of the aglycone. It may link to the sapogenin through an ether or ester linkage at one or two glycosylation sites. Attachment of the aglycone to three sites (tridesmosidic) in a sapogenin is rare [26]. Many saponins are present in higher plants in the form of glycosides of complex alicyclic compounds and show characteristic foaming properties in aqueous solution. The triterpenes are subdividedinto 20 groups, depending on their particular structures. The base structure found in the largest variety of medicinal plants is the oleananetype triterpene. In fact isolated oleanolic acidis a substance of therapeutic interest [27].

Up to present day 1730 species of 104 families of plants growing in the Central Asia found tritepenoid saponins were present in 627 species and steroidal saponins in 127 species [28]. About 76% of plant families were examined saponins, suggesting a wide distribution of saponins in the plant kingdom. The plant saponin have been used for medical purposes such as oriental drugs, many herbal drugs contain saponin [29, 30]. Saponins are important for therapeutic drugs such as cortisoneand contraceptive oestrogens. Pharmacological activities of saponins are cytotoxicity, anti-tumor, anti-mutagenic, anti-inflammatory, anti-viral and cardiac activities , in addition to anthelmintic activity[30].

Steroids: In the present study steroids was screened from eleven plant species and showed that ten species were reported low level of steroids and one species reported moderate level of steroids in *Terminalia chebula* (Retz).

Steroids were first observed in the 1930's. The Germans first experimented on dogs and then on their own soldiers in the World War II, and on their prisoners to help them stay healthy because they suffered from significant malnutrition. Then in the 1950's many Russian and European athletes began to find that steroids were very beneficial to their goals and soon after began dominating the sport of power lifting, crushing previous world records. In the mid 1950's it was showed that testosterone was the reason behind the improved athletic ability. A few years later, steroids were available on the market and athletes and doctors were using them alike on a regular basis, making them an illegal substance without a prescription. Today, there are clinics that will prescribe testosterone and HGH (Human Growth Hormone) to qualified patients.

Hundreds steroids are found in plants, animals and fungi. All steroids are made in the cell from sterols lanosterol in animal and fungi and from cycloartenol in plants. Both are derived from cyclization of the triterpenesquallene [31]. These are metabolites and functions as signalling molecules and along with phospholipids function as components of cell membranes and cholesterol decreases membrane fluidity [32].

Tannin: It was reported from the screened data of eleven species of plants eight plant species were reported tannin in moderate and high level in *Terminalia chebula* (Retz) and *Vitex nigundao* (L) while three species were not reported tannin content.

Tannins are antinutrients and are used in the astringent taste of foods and drinks [33]. Tannins bind to proteins and carbohydrates which has several implications for commodities containing tannins. Their presence can cause browning or other pigmentation problems in fresh foods and processed products. The presence of tannin in the plants is useful for the healing of wounds and burns [34]. Tannins and phlobatannins had been reported to have wound healing properties.

Quinine: In the present task eleven plant species were screened for quinine and reported that six plant species showed high content, moderate in three species and low in two species tested.

Quinine occurs naturally in the bark of the cinchona tree, though it has also been synthesized in the laboratory. The medicinal properties of the cinchona tree were originally discovered by the Quechua, who are indigenous to Peru and Bolivia; later, the Jesuits were the first to bring cinchona to Europe.

Today there is a wide range of medicinal plant parts which include the flowers, leaves, stem, fruits and root extracts which are used as powerful raw drugs possessing a variety of antimicrobial and healing properties. The phytochemical showed the presence of secondary metabolites including phenols, saponins, tannins and coumarins which had great medicinal properties. There are several reports to showed antimicrobial chemicals [35].

The medicinal plants are rich in secondary metabolites which include alkaloids, flavonoids, steroids and related active metabolites which are of great medicinal value and have been extensively used in the drug and pharmaceutical industry. Recently number of studies had been reported on the phytochemistry of medicinal plants, particularly on the vegetative parts like leaves and stems etc. [36, 37, 38]. Phytochemicals especially plant phenolic constitutes a major group of compounds that act as primary antioxidants. The phenolic compounds have high redox potential which allow them act as reducing agents, hydrogen donors and singlet oxygen quenchers [39]. The antioxidant effects of the extract may be due to its phenolic content. The delocalization of electrons over the phenolic and stabilization by the resonance effect of the aromatic nucleus prevents the continuation of the free radical chain reaction. The DPPH assay has been largely used as a quick, reliable and reproducible parameter to search for the *in vitro* general antioxidant activity of pure compounds as well as plant extracts [40]. The decrease in absorbance by the DPPH radical with increase in concentration of the extract which manifested in the rapid discolouration of the purple DPPH, suggest that theantioxidant activity due to its proton donating ability. The extract was found to highly scavenge free radicals when compared to standard antioxidants. The reducing capacity of compounds could serve as indicator of potential antioxidant properties and increasing absorbance could indicate an increase in reducing power. Nitric oxide is a free radicals product and involved in the regulation of various physiological processes, however excess production of nitric oxide is associated with several diseases [41].

Antibacterial activity: The results of screening plant extracts against antibacterial activities are presented in table 4. The methanol extract of eleven plant species belonging to ten families were tested against three gram-positive and negative bacterium using agar well diffusion. The plant exhibited antibacterial activities to a certain degree. *Terminalia chebula* (Retz) followed by *Glycyrrhiza glabra* (L) showed highest zone of inhibition in gram positive and negative bacterium.

Out of eleven species tested none of the species were totally active in al bacteria strains evaluated. The antibacterial activity varies species to species because of the antibacterial substances presentin plant. There is need to successful evaluation of plant substances and the type of solvent used in the extraction procedures [42] Researcher mostly preferred water extract, but extracts in organic solvent (methanol) provided more consistent antibacterial activity compared to water. Several workers have identified plant compounds that are known to be antibacterial [43]. Traditional herbal remedies used in world are important sources for discovery of new antibiotics [45]. The antibiotic property of plant compounds that indicates the need for further search in to traditional system but need to reduce possible toxicity present in plant compound.

The plant extracts did not showed antibiotic activity, but negative result does not mean absence of bioactive compounds nor is that the species inactive. Active compounds may be present in insufficient quantities in the extract to show the activity [46]. Lack of activity can thus only be proved by large dose level and the active compound present in high enough quantities'. It is also showed, those gram-positive bacteria are more sensible than gram-negative because due to single layer cell wall in gram-positive bacterium. There are variations in antibacterial activities in species to species due to different phytochemicals in species to species.

Antifungal activity: A differences observed in antifungal activity of methanol extracts of eleven species against four fungal strains tested (Table 4). Among the eleven species from ten families were screened observed significant antifungal activities and varies species to species. The species *Termanalia chebula* (Retz) showed highest zone of inhibition in *Aspergillus niger* and *Trichoderma viridae* strains followed by *Vitex nigundo* (L) and *Bacopa mannier* (L). A lower zone of inhibition was reported from *Glycyrrhiza glabra* (L) and *Dioscor bulbifera* (L).

The present investigated species extracts did not showed any antifungal activity, it does not mean absence of antifungal bioactive compounds. It may be in trace quantity and not reflect the activity. The activity may be proven by using large dose or high enough quantities or antagonistic effects [47]. Plant based antifungal activity is also better for pest management. Due to pesticide pollution and its side effects on biotic and abiotic, it is alternative to chemical pesticides.

Cytotoxicity: Eleven plant species (table 6) were selected for their establishment and three species were found high percent viability such as *Terminalia chebula* (Retz) (72.2%), *Acacia catechu* (L) (65.8%) and *Chlorophytum borivilianum* (L) (59.9%).The fibroblast cell line was grown successfully. Methanol extract (control) shows the more percent viability than other concentrations. Less growth was observed in 20µl concentration of sample. Cytotoxicity is the quality of being toxic to cell. Treating cells with the cytotoxic compound can result in a variety of cell fates. The cells may undergo necrosis, in which they lose membrane integrity and die rapidly as a result of cell lysis [7].

Animal studies involving rats and mice, as well as *in vitro* studies utilizing human cell lines, have demonstrated *Curcuma amada* rhizome extract ability to inhibit carcinogenesis at three stages astumour promotion, angiogenesis [48] and tumour growth [49] (Limtrakul et al., 1997). The disease colon and prostate cancer, from *Curcuma amada* Rhizome extract inhibited cell proliferation and tumour growth [50,51]. This present work described the chick embryo cell line fibroblast toxicity on control and induced cells. Observation was made on eleven selected plant extract on fibroblast. In evaluated twenty plant species and were found three species high per cent viability such as *Tinospora cardifolia* (82.33%), *Plumbago zeylanica* (76.66%) and *Withania somnifers* (69.81%). The viability varies species to species due to ingredient contents in the species [7].

IV. CONCLUSION

Since bioactive compounds occurring in plant material consist of multicomponent mixtures, their separation and determination is most important. The bio-components most of plants have to be purified and isolated and used in theraptic use are most important. The alkaloid and flavonoid content of plant materials has severally been reported to be a major antioxidant, anti-inflammatory and analgesic active principle; while tannins and phlobatannins has been reported to have wound healing properties.

REFERENCES

[1] Singh, A. P. 2005. Promising phytochemicals from Indian medicinal plants. *Ethnobotanicals leaflets,* **1**(1), Article 18.

[2] Madhuri, S. and Pandey, G. 2009. Some anticancer plants of foreign origin.*Curr. Sci.* **96**, 6.

[3] Singh, R., Singh, M. K., Chandra, L. R., Bhat, D., Arora, M. S., Nailwal, Y. and Pande, V. 2012. Antioxidant and free radical scavenging activity of *Macrotyloma uniflorum* (Gahat dal) from Kumauni region. *Int. J. Fundam. Appl. Sci*. **1**, 7-10.

[4] Aruoma, O. I. 2003. Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. *Mutation Res.* **9**, 523-524.

[5] Goyal, A. K., Basistha, B.C., Sen, A. and Middha, S.K. 2011. Antioxidant profiling of *Hippophae salicifolia* growing in sacred forests of Sikkim, India. *Funct. Plant Bio.* **38**, 697-701.

[6] Middha, S. K., Mittal, Y., Usha, T., Kumar, D., Srinivasan, R., Vashisth, L., Bhatacharjae. B. and Navaveni, M. B. 2009. Phytomellitus: A phytochemical database for diabetes. *Bioinformation*, **4,** 78-79.

[7] Kakad, S. L. and Dhembare, A. J. 2014. The cytotoxicity of different plant extract on chick embryo fibroblast cell line. *Archives of Appl. Sci. Res*. **6** (4), 139-142.

[8] Kakad, S.L., Pise, S.S. and Dhembare, A. J. 2015a. Evaluation of phytochemical, antibacterial, antifungal activities of leaf extracts of *Moringa citrifolia* (Linn). *Der Pharmacia Sinica*, **6** (4), 9-12.

[9] Kakad, S. L., Dhembare, A. J. and Ruchita Chakane., 2015b. Evaluation of antifungal activities of some selected plant species against fungal pathogen. *J. Microbil. Biotech. Res.* **5**(1), 24-27.

[10] Kakad, S.L., Tungikar, V.V. and Dhembare, A. J. 2015c. Evaluation of antibacterial activity of plant extracts against bacterial pathagon. *Der Pharmicia Sinica* , **6** (1), 1-5.

[11] Wagner, H. and Bladt, S. 1996. Plant Drug Analysis. II nd Ed., *Berlin, Springer*, **2**(1), 349-354.

[12] Quettier-Deleu, C., Gressier, B., Vasseur, J., Dine, T., Brunet, J., Luyck, M., Cazin, M., Cazin, J. C., Bailleul, F. and Trotin, F. 2000. Phenolic compounds and antioxidant activities of buckwheat (*Fagopyru mesculentum* Moench) hulls and flour. *J. Ethnopharmacology***, 72**, 35-40.

[13] Polya, G. M. 2003. Biochemical targets of plant bioactive compounds a pharmacological reference guide to sites of action and biological effects. CRC Press, Florida.

[14] Kagbo, H. and Ejebe, D. 2009. Phytochemistry and preliminary toxicity studies of the methanol extract of the stem bark of *Garcinia kola* (Heckel). *Internet J. Toxico*.,**7**(2),1-13.

[15] Li, F. K., Lai, C. K., Poon, W. T., Chan, A. Y. W., Chan, K. W., Tse, K .C., Chan, T. M. and Lai, K. N. 2004. Aggravation of non-steroidal anti-inflamatory drug-induced hepatitis and acute renal failure by slimming drug containing anthraquiones. *Nephrol Dial Transplant*, **19** (7), 1916-1917.

[16] Yen, G. C., Duh, P. D. and Chuang, D. Y. 2000. Antioxidant activity of anthraquinones and anthrone. *Food Chem*. **70** (4), 437-441.

[17] Sendelbach, L. E. 1989. A review of the toxicity and carcinogenicity of anthraquinone derivatives. *Toxicology*, **57** (3), 227-240.

[18] Alves, D. S., Pérez-Fons, L., Estepa, A. and Micol, V. 2004. Membrane related effects underlying the biological activity of the anthraquinones, emodin and barbaloin. *Biochem. Pharmacol.*, **68** (3), 549-561.

[19] Bolhius, G.G. 1954 . The toxicity of cassava roots. *Neth. J. Agric*. **2,** 176-185.

[20] Terashima, K., Takaya, Y. and Niwa, M. 2002. Powerful antioxidative agents based on garcinoic acid from garcinia kola. *Bioorg Med Chem*, **10** (5), 1619-1625.

[21] Okwu, D. E. 2004. Phytochemicals and vitamin content of indigenous species of South Eastern Nigeria. *J. Sustain Agric. Environ*. **6**, 30-34.

[22] Akinmalodun, A.C., Ibukun, E.O., Afor, E., Akirinlola, B.L., Onibon, T. R., Akinboboye, A.O., Obuotor, E. M. and Farombi, E.O. 2007. Chemical constituents and antioxidant activity of *Alstoni aboonei. Afr. J. Biotechnol*. **6** (10), 1197-1201.

[23] Eleazu, C. O., Okafor, P.N., Amajor, J., Awa, E., Ikpeama, A. I. and Eleazu, K. C. 2011. Chemical composition, antioxidant activity, functional properties and inhibitory action of unripe plantain (*M. paradisiacae*) flour. *Afr. J. Biotechnol*. **10** (74), 16948-16952.

[24] Liang, Y. C., Huang, Y. T., Tsau, S. H., Lin-Shiau, S. Y., Chen, C. F. and Lin, J. K. 1999. Suppression of inducible cyclooxygenase and inducible nitric acid synthase by apigenin and related flavonoid in mouse macrophages. *Carcinogenesis*, **20,** 1945-1952.

[25] Elekwa, O. K. 1985. Prevention of thiacetimide induced hepa-totoxicity by Kolaviron. B. Pharm Thesis. Department of Pharmacology University of Nigeria, Nsukka.

[26] Natori, S., Ikekawa, N. and Suzuki, M. 1981. Advances in Natural Products Chemistry: Extraction and Isolation of Biologically Active Compounds, Kodansha Ltd, Tokyo112, Japan, pp 275- 287.

[27] Liu, J. 1995. Pharmacology of oleanolic acid and ursolic acid. *J. Ethnopharmacology*, **49**, 57-68.

[28] Long, Q. Q. 1989. Total saponin contents of the root of wild and cultivated *Platycodongr andiflorum. J. Chinese Medicinal Materials*, **12** (3), 37-38.

[29] Lacaille-Dubois, M. A. and Wagner, H. A. 1996. A review of the biological and pharmacological activities of saponins. *Phytomedicine*, **2**(4), 363-386.

[30] Osamu, T. 1990. Ginseng saponins from Panax species. *Pure and Appl. Chem*, **62** (7), 128-1284.

[31] Lednicer, D. 2011. Sterois Chemistry at a Glance. Hoboken: Wiley ISBN 978-0-470-66084-3

[32] Sadava, D., Hillis, D. M., Heller, H.C. and Berenbaum, M. R. 2011. Life; The science of biology, 9thEd. San Francisco: Freeman. pp 105-114.

[33] Chikezie, P.C., Agomuo, E.N. and Amadi, B. A. 2008. Biochemistry, Practical/Research Method, A Fundamental Approach. Vol 2, *Mega soft publishers*, p. 51-53.

[34] Farquar, J. N. 1996. Plant sterols, their biological effects in human. Handbook of Lipids in Nutrition BOCA Rotan HL CRC Press. pp. 101-105.

[35] Kannan, D., Mehra, R. S., Dubey, S., Tiwari, S., Maheshwari, U. and Bisht, V. S. 2013. Evaluation of phytochemical constituents, antibacterial activities, cytopathic and cytotoxic effects of extracts of *Tylophora indica, Curcuma amada* and *Urticadioica*. *J. Recent Adv. Appl. Sci.*, **28** (1): 1-11.

[36] Balakumar, S., Rajan, S., Thirunalasundari, T. and Jeeva, S. 2011. Antifungal activity of *Aeglema rmelos* (L) Correa (Rutaceae) leaf extract on dermatophytes. *Asian Pacific J. Trop. Biomed.*, **1**(4), 309-312.

[37] Paulraj, K., Irudayaraj, V., Johnson, M. and Patric, R. D. 2011. Phytochemical and antibacterial activity of epidermal glands extracts of *Christellaparasitica* (L) H. Lev. *Asian Pacific J. Trop. Biomed.* **1**(1), 8-11.

[38] Kala, S., Johnson, M., Raj, I., Bosco, D., Jeeva, S. and Janakiraman, N. 2011. Preliminary phytochemical analysis of some medicinal plants of South India. *J. Natura Conscientia*. **2**(5), 478-481.

[39] Kahonen, H.N., Kohonen, M., Wu, X., Sand, J., Nordback, I., Taurio, J.U. and Porsti, I. 1999. Control of vasculat tone in isolated mesentric arterial segment from hypertensive patients. *Br. J. Pharmacol*. **127**, 1735-1743.

[40] Koleva, I. I., Van Beek, T. A., Linssen, J. P. H., de Groot, A. and Evstatiena, L. N. 2002. Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. *Phytochemical Analysis*, **13**, 8-17.

[41] Lalenti, A., Moncada, A. and De Rosa, M. 1994. Modulation of perspective for the 1990s. *Nature*, 234, 462.

[42] Srinivasan, D., Nathan, S., Suresh, T. and Perumalsamy, D. 2001. Antioxidant and antibacterial activity of different parts of *Leucas aspera*. *J. Enthnopharmacol*. **74**, 217-220.

[43] Basil, A., Sorbo, S., Giordano, S., Ricciardi, L., Ferrara, S., Montasano, D., Castaldo, R., Cobianchi, M., Vuoto, L. and Hocquemiller, R. 2000. *Fitoterapia* , **71**, 110-116.

[45] Okpekon, T., Yolou, S., Gleye, C., Roblot, F., Loiseau, P., Bories, C., Grellier, F., Frappier, F., Laurens, A. and Hocquemiller, R. 2004. Antiparasitic activities of medicinal plants used in Ivory Coast. *J. Ethenopharmacol***, 90**, 91-97.

[46] Taylor, L.J.S., Rabe, T., McGrew, L. J., Jagar, A. K. and Van Steden, J. 2001. Towards the scientific validation of traditional medicinal plants. *Plant Growth Regul*. **43**, 23-37.

[47] Jager, A.K., Hutchings, A. and Van Staden, J. 1996. *In Vitro* Antimicrobial activity of extracts from plants used traditionally in South Africa to treat Tuberculosis and related symptoms. *J. Ethenopharmacol.* **52,** 95-100.

[48] Thaloor, D., Singh, A. K. and Sidhu, G. S. 1998. Inhibition of angiogenic differentiation of human umbilical vein endothelial cells by curcumin. *Cell Growth Differ*, **9**(2), 305-312.

[49] Limtrakul, P., Lipigorngoson, S. and Namwong, O. 1997. Inhibitory effect of dietary curcumin on skin carcinogenesis in mice. *Cancer Lett*, **116** (23), 197-203.

[50] Hanif, R., Qiao, L., Shiff, S.J. and Rigas, B. 1997. Curcumin, a natural plant phenolic food additive, inhibits cell proliferation and induces cell cycle changes in colon adenocarcinoma cell lines by a prostaglandin independent pathway. *J. Lab. Clin. Med*. **130** (2), 576-584.

[51] Dorai, T., Cao, Y.C. and Dorai, B. 2001. Therapeutic potential of curcumin in human prostate cancer. III. Curcumin inhibits proliferation, induces apoptosis, and inhibits angiogenesis of LNCaP prostate cancer cells in vivo. *Prostat*, **47** (11), S293-303.

