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Bioaasy of Two Indian Medicinal Plants Bark Extracts on Seed Germination of *Arachis hypogea and Oryza sativa*- as Potential Herbicide.

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ABSTRACT

Phytochemicals are extensively found at different levels in many medicinal plants. This work had two objectives: The first, to evaluate the Total phenolic (TP) and Total flavanoid contents(TF) of two Tradiational Indian medicinal plants and second to determine whether these compounds have an Germination and Growth inhibition capacity towards Dicotyledonous Arachishypogea and Monocotlydenous oryza sativa plant Propagation. The polyphenolic extraction of the dried powdered bark samples have been performed using (70% Ethanol and 30% distilled water) Hydro-alcohol. The total phenolic content , analyzed using Folin-ciocalteu's reagent were found to be 32.15 and 20.38mg/g dry weight, expressed as Gallic acid equivalents(GAE). The total flavanoid concentrations, detected using 2% Alluminium chloride, were found to be 13.2, and 7.46mg/g Rutin Equivalents (RE) of combined hydro-alcoholic bark extract. With further data analysis it was found that there was a positive correlation between the total phenolic, total flavanoid content of bark extract and its growth inhibition activity was determined to be R^2=0.9968.This also suggests that phenolics and flavanoids in these plant provide substantial growth inhibitory activity. Upon achievement of this survey, and using more plants, an extra benefit of these medicinal plant may be found. Flora of India appears to be a rich and interesting source for supplementary Ethnomedicinal and phytochemical studies .

Key words: Medicinal plants; Total phenolic content; Total flavanoids content; Growth inhibition; Bioassay

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INTRODUCTION

The need to reduce harmful environmental effects from the over use of chemicals has encouraged the development of weed management systems , which are dependent on ecological manipulations rather than agrochemicals[1]. Allelopathy has been defined as an adverse influence of one plant or microorganism on another [2]. In agricultural practice allelopathy is exploited for weed control[3]. Numerous plants have been shown to have strong allelopathic activity, out of them two commonly known medicinal plant were selected for allelopathic study[4]. A variety of plants have been reported to possess allelopathic activity on the growth of other plant species compound with allelopathic activity are present in many plant and in many organs, including leaves, stems, flower, bark etc.[5,6].There are hundreds of secondary metabolites in the plant kingdom and many are known to be phytotoxic [7].Allelopathic effect of these compound are often observed to occur through out the plant life cycle causing inhibition of seed germination or seedling growth[8]. One of the most studied aspects of allelopathy is its role in agriculture [9,10,11]. Forest species produce large amounts non-wood forest products in which bioactive substances are present in high percentages. These compounds can be transported to the ground by exudation from roots or

leaching of arieal parts. The increased forest area is not only a source of wood as forestry systems provide an excellent opportunity to explore the properties of these species in the control of weeds, insects and nematodes or for the improvement of grounds and as a source bioactive products in pharmacology[12].

Tectona grandis (Teak), family verbenaceae is a native tree from tropical countries of Asia. Teak wood is one of the most valuable and better known woods and it has a Large number of applications in the timber industry due to its beautiful surface and its resistance to termite and fungal damage[13]. Research on this plant has held to interest in terms of alleopathy because teak has been successfully used in agro forestry system and in crop rotation in India[14]. During the second half of 19^{th} century , the increasing demand for teak wood led to the implantation of the agro forestry system denominated in India and other tropical countries in Asia. This consists of cultivating maize within young teak plantations[15]. Since then ,this species has been used successfully in culture rotation and is combined with agricultural species such as cotton, chilli, ginger. In India it was found that teak plantations with peanut and rice were unsuccessful and negative effects on the growth of teak were not found[16]. Later studies proved the alleopathic effect of leaves on the germination of peanut and maize[17]. Tectona grandis has also shown high alleopathic activity on wheat[18,19].

Azadirachta indica (A.Juss), family Meliaceae popularly known as neem (English). The plant is perhaps one of the most studied and widely used medicinal plant[20]. The species is presently being cultivated world wide because of its ability to adapt to different climatic conditions. Biological and pharmacological activities attributed to solvent extracts and products like oil from the different parts of A. indica are as diverse as Antiplasmodial, Larvicidal, Fungicidal, Insecticidal, Anthelminthic[21]. Further more, the bark of neem tree is known to posses tannins,and phenolic compounds and exhibits anti-oxidant principles[22]. Literature survey provided information; when about 250 species of trees were screened for allelopathy in Japan by using a sandwich method it was reported that neem leaves strongly exhibits growth inhibitory action[23]. Although much research has been done on neem in different aspects little information is available concerning the allelopathic potential of neem on crop plants[24]. In order to study the potential use of two medicinal plants as a source of natural herbicide model and /or bioactive compounds we put a step forward and report here in detail the systematic Alleopathic influence and phytochemical studies on combined Hydro alcoholic bark extract of Azadirachta indica and Tectona grandis on germination and growth inhibition of *Arachis hypogea* and *oryza sativa.*

Aim and Objective

The aim of present Research work is to demonstrate the natural Herbicidal effect of two important Indian Medicinal plants bark extracts ie. the study of Allelopathic effect in the laboratory using coarse soil as a medium for growth of seeds.The plant extracts were given to the germinating seeds at regular intervals for about 72 hrs of observation time period and the herbicidal effect was reported.

MATERIALS AND METHODS

2.1. plant materials

The stem bark of *Azadiractha indica* (Meliaceae) and Tectona grandis (verbanaceae) were collected within the college premisis Hindu college of pharmacy Amaravathi road, Guntur during November 2012. The plant materials were identified and Authenticated by Prof.G.Diwakar Department of Botany ,Acharya Nagarjuna university, Guntur, A.P, India(Ref.no.ANU/WC/B569,B570/2013).

2.2 Preparation of Extract

The plant materials were cleaned, dried under shade cut into pieces pulverized together by using grinder. The powder of plant (500g) was extracted with petroleum ether and then with hydro alcohol, composition 70:30 ml ratio of ethyl alcohol and distilled water respectively using soxhlet apparatus for 72h. This was filtered with whattman filter paper No.1 and the brownish coloured filtrate was concentrated at 40^0C using a Rotary evaporator (Laborota 4000-Efficient, Heidolph, Germany). which was stored in Refrigerator maintained at 4^0C. The yield of extracts obtained with petroleum ether was 1.3% and Hydro alcohol 16.63%.Hydro alcohol was used in the present research study because it was the most frequently Mentioned vehicle for this particular plants during our Ethno botanical Literature survey. preliminary phytochemical study revealed the occurrence of Sterols, Flavanoids, Alkaloids, Tannins, phenolics in the extracts.

Table-1

Plant extract	Total phenol Phenolic conten (mg GAE/g dry weight)	Flavanoid content (mg RE/ g dry weight)
Azadiractha indica	32.12± 0.2	13.12±0.1
Tectona grandis	20.38± 0.30	7.46 ± 0.20

2.3 Chemicals and Reagents

All the chemicals were purchased from sigma (USA) Aldrich (Mil Waukee, USA), Fluka chemie (Buchs, Switzerland) and Merck (Germany).

2.4 Extraction of phenolics:

The air dried plant material (1g) was crushed and extracted for 24hr with 50ml of 70%. (v/v) aqueous ethanol at room temperature. After removal of ethanol under reduced pressure in a rotary evaporator at 40^0C, the remaining aqueous solution of the extraction was extracted with ethyl acetate, in the presence of aqueous solution with 20% Ammonium sulfate, and 2% of meta-phosphoric acid solution. The ethyl acetate fraction was dried by adding a sufficient amount of anhydrous sodium sulfate, and then evaporated. The precipitate was dried, dissolved in 5ml of absolute methanol and kept at 20^0C.

2.5 Estimation of total phenolic content

The amount of total phenolics was determined with Folin – ciocalteu reagent using the method of lister and Wilson[25]. This method was employed to evaluate the phenolic content of the samples .A standard curve must first be plotted using Gallic acid as a standard. Different concentrations of Gallic acid were prepared in 80% of methanol and their absorbance were recorded at 765nm.100µL of sample was dissolved in 500µL(1/10dilution) of the folin-ciocalteu reagent and 1000µL of distilled water. The solutions were mixed and then incubated for 2hrs in the dark at room temperature. The absorbance of all samples was measured at 760nm using a shimadzu UV-Vis spectrophotometer. Calibration cured for gallic acid (10-100ppm) (R^2=0.9986), and the results are expressed in mg of gallic acid per [GAE] dry weight of plant.

2.6 Estimation of flavanoids content

The flavanoids content in extracts was determined spectrophotometrically according to Lamaison and carant standard procedure [26]. The method based on the formation of a complex Flavanoids- Alluminium ,having the absorbtivity maximum at 430nm. Rutin was used to make the calibration curve. 1mL of diluted sample was separately mixed with 1mL of 12% Alluminium chloride methanolic solution. After incubation at room temperature for 15min, the absorbance of the reaction mixture was measured at 430nm with a shimadzu UV-Vis spectrophotometery and the flavanoids content was expressed in mg per gm of Rutin equivalent [RE].

Table-2

Preliminary	phytochemical study of Azadiractha indica	Hydro alcoholic bark extracts Tectona grandis
Test for sterols	√	√
Test for Glycosides	×	×
Test for Alkaloids	√	√
Test for Flavanoids	√	√
Test for Phenolic	√	√
Test for Dyes	×	√

√: Present *x: Absent*

2.7 Statistical analysis

Experimental data was subjected to one way analysis of variance (ANOVA) Followed by Duncan's multiple range test to determine significance differences among mean values at the probability level of 0.05 statistical analysis. ANOVA test was used to determined the level of significance within the *Arachis hypogea* and *Oryza sativa* regarding the effect of combined hydro alcoholic bark extract on germination and seedling growth, significance of difference was accepted when $P \leq 0.05$.

2.8 Bioassay

2.8.1 Germination and seedling growth test

Twenty five seed of *Arachis hypogea* and *oryza sativa* were germinated separately in petridishes on filter paper with 10 mLof each concentration of Hydro alcoholic bark extract of Azadiractha indica and Tectona grandis. Distilled water was used as control. Three replicates were incubated in a Randomized complete block design at 20^0C in an incubator with fluorescent light. Germination criteria were the emergence of the radical through the pericarp. Germination percentages were recorded every day and total seedling length was measured after 3 days of incubation using five seedling taken randomly from each dish. The results were determined by counting the number of germinated seeds and measuring the length of root and shoot on 3rd day of the experiment. The data were subjected to analysis of variance and Duncan's multiple range test. Ratio of germination and elongation were calculated as suggested by Rao and Kil [27].

$$\text{Relative Germination ratio} = \frac{\text{Germination ratio of tested plant} \times 100}{\text{Germination ratio of control}}$$

$$\text{Relative elongation Ratio of shoot} = \frac{\text{Mean shoot length of tested plants} \times 100}{\text{Mean shoot length of control}}$$

$$\text{Relative elongation Ratio of root} = \frac{\text{Mean root length of tested plant} \times 100}{\text{Mean root length of control}}$$

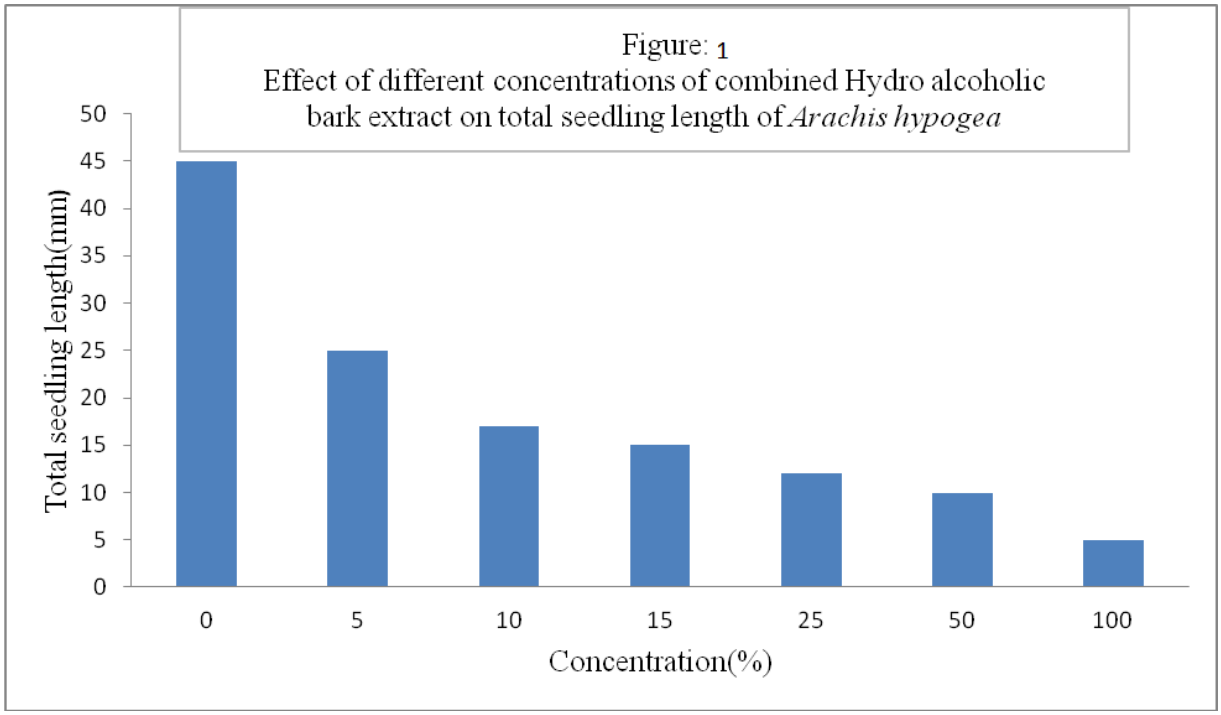


Figure:2
Effect of different concentrations of combined Hydro alcoholic barextract on total seedling length of oryza sativa

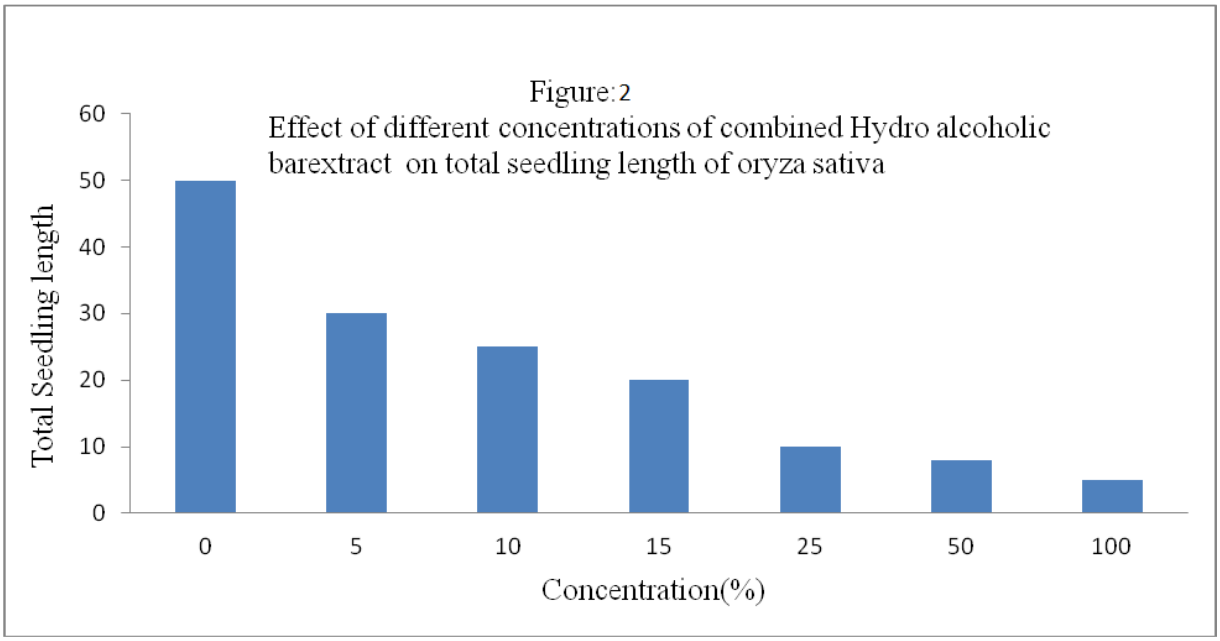


Figure: 3
Relative comparision of the effect of different concentrations of combined Hydroalcoholic bark extract on shoot and root length of *Arachis hypogea*

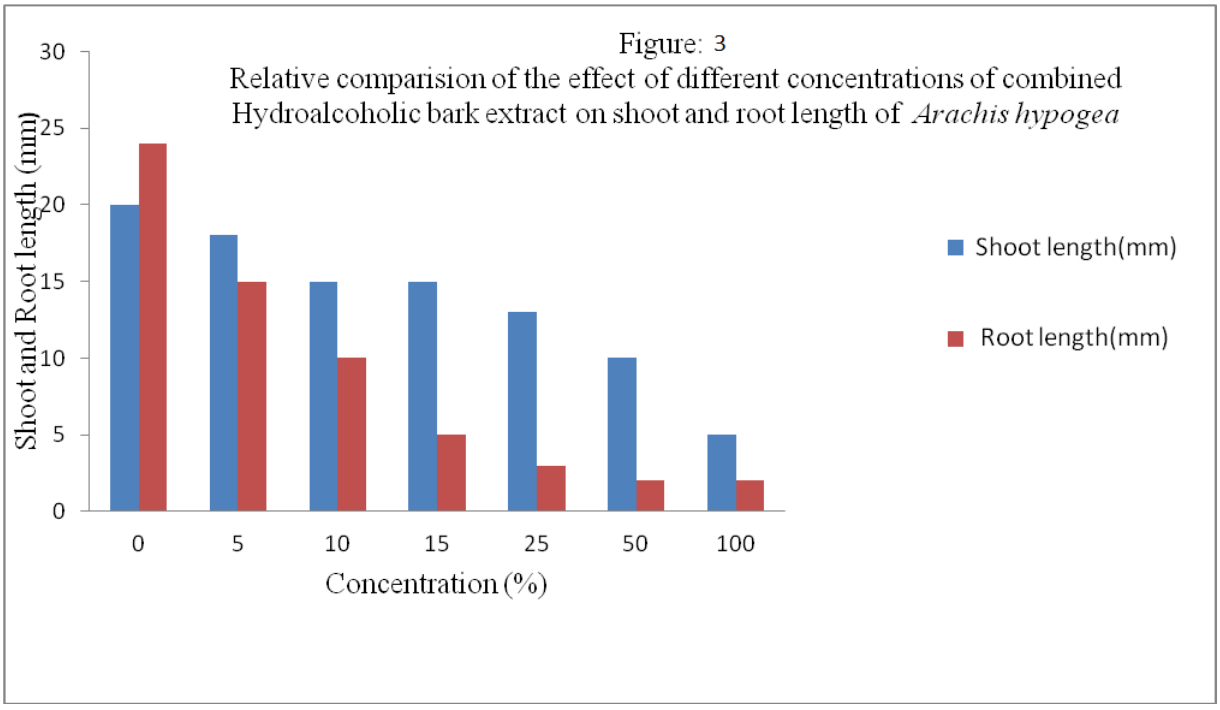


Figure: 4

Relative comparision of effect of different concentrations of combined Hydro alcoholic bark extract on shoot and root length of *Oryza sativa*

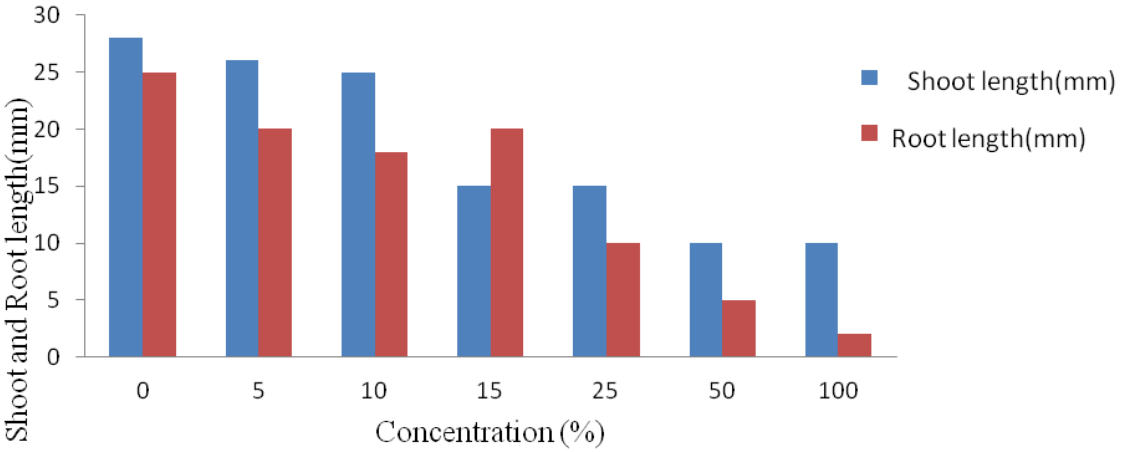
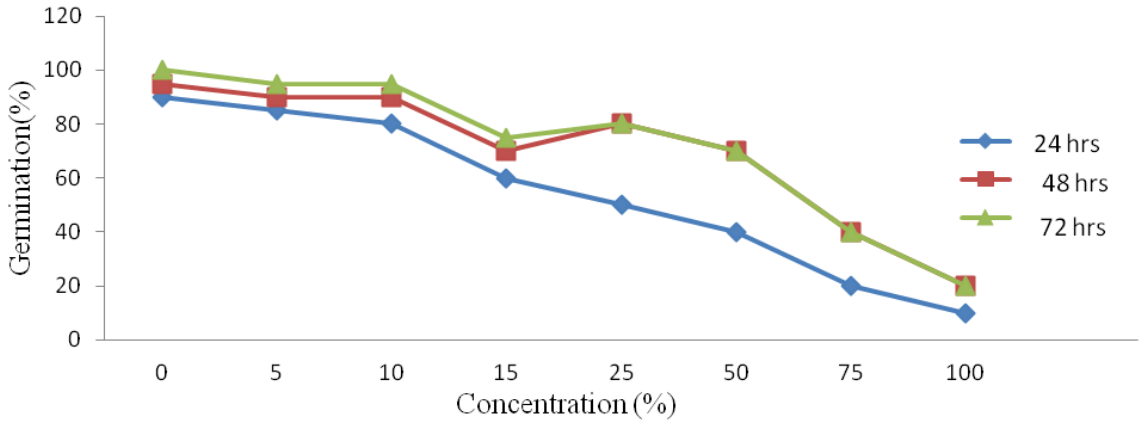
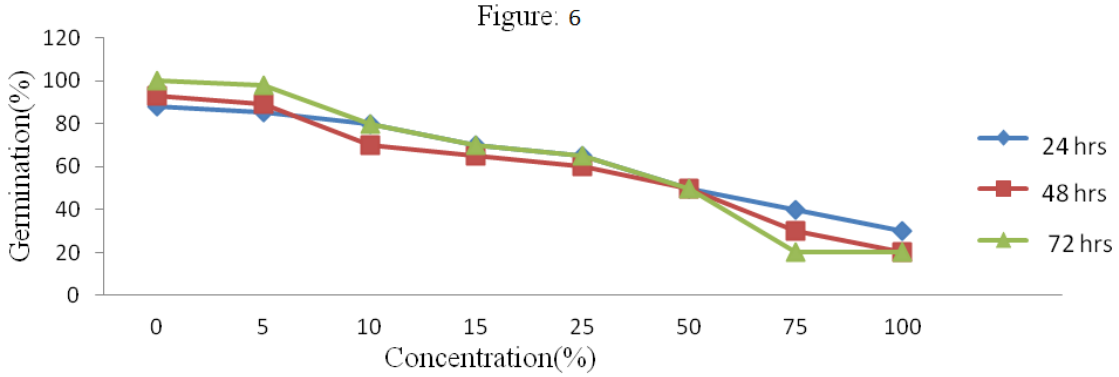


Figure: 5



Relative germination ratio of bioassay species grown in petridishes at differentconcentrationof combined Hydro alcoholic Bark extract of *Azadiracthaindica*,*Tectonagrandis* on*Arachishypogea* seeds.

Figure: 6



Relative germination ratio of bioassay species grown in petridishes at different concentration of combined Hydro alcoholic Bark extract of *Azadiractha indica, Tectona grandis* on *Orayza sativa* seeds.

RESULTS AND DISCUSSION

The amount of total phenolics estimated in combined Hydro alcoholic extract was found to be 32.3 mg in Azadiractha indica and 20.38 mg in Tectona grandis measured as GAE/g of dry material respectively. From analysis we can deduced that these two plant are rich in flavanoids. We outline that the amount of flavanoids in the bark of plants were 13.12 mg and 7.46 mg /g rutin equivalent of the crude extract of *Azadiractha indica* and Tectona grandis respectively. However, we can state as sugars, proteins and pigments which may interfere during the total phenolic evaluation upon such extraction, we have obtained results which shows a significant high total amount of the phenolics. We also mention here that an increase of the phenolic metabolism in these saharian plant may be related to the hard climate conditions (Hot temperatures, high solar exposure, dryness, long life growing season).The result suggests that 79% of the Growth Inhibition capacity of Indian medicinal plant is due to the contribution of phenolic and flavanoids compounds. Also it can be concluded that the growth inhibition activity of plant extracts is not the result of these compound but may be also related to the presence of some individual active phenolic compounds .The unclear relationship between the growth inhibition activity and the total phenolic may be explained in numerous ways ,in fact the total phenolic contents does not in corporate all the inhibition. In addition, the synergism between the content in the mixture such as flavanoids, phenolics makes the growth inhibition. The purpose of this study was the evaluation by a bioassay method on the germinated *Arachis oryzasativa* seeds to demonstrate the inhibitory nature of phenolic and flavanoids compounds in Indian medicinal plants. Germination percentages of *Arachis hypogea* and *Oryza sativa* after all periods of inhibition were decreased as the concentration of hydro alcoholic bark extract of *Azadiractha indica* and Tectona *grandis* increased, but the inhibition of germination was more pronounced ($P<0.05$) after first day of imbibitions. The seedling growth either total seedling length[Fig. 1, Fig. 2], Root and shoot length of green peas and paddy grains[Fig-3,Fig-4] also decreased with increasing concentrations of extract in comparision with water control. The bark extract had significant ($P\leq 0.05$) toxic inhibitors on root and shoot length of germinated seeds. Germination percent of receptor plant seeds to distill water and effect of and different concentration of extracts values indicates the stimulatory (or) inhibitory effects in comparision to control[Fig-5,Fig-6]

CONCLUSION

Since the prehistoric era, herbs have been the basis for nearly all medicinal therapy as well as Agroforestry cultivation such as pesticide, herbicide,etc until synthetic drugs & Agrochemicals were developed in the nineteenth century.Allelopathy as a mechanism of plant interference in Agro-ecosystems offers opportunity to manage weeds in a crop sequence, but could also adversely affect crop yields and influence choice of rotation. Chemicals with allelopathic activity are present in many plants.This research furthers the possibility of using allelochemical as growth regulators and Natural pesticides, a number of them are either commercially available or in the process of Large scale Manufacture to promote sustainable agriculture.

We propose that the extracted Flavanoids& Phenolic compositions in the Hydro alcoholic bark extract were involved in the strong inhibition action of growth regulators in the germinated seeds.

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