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Evaluation of bioactive compounds from selected medicinal plant extract as root promoter potential

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By

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Bhubaneswar
Date: 13.9.21

CERTIFICATE- I

This is to certify that the thesis entitled **"Evaluation of bioactive compounds from selected medicinal plant extract as root promoter potential"** submitted in partial fulfillment of the requirements for the award of the degree of **Master of Science in Agriculture (Agricultural Biotechnology)** to the Odisha University of Agriculture and Technology is a faithful record of *bona fide* and original research work carried out by **Ms. Trupti Dash (Adm. No. 191221210)** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help obtained by her from various sources during the course of investigation has been duly acknowledged.

GRRout 13/09/2021

(Dr. Gyana Ranjan Rout)
Chairman
Advisory Committee

CERTIFICATE-II

This is to certify that the thesis entitled **"Evaluation of bioactive compounds from selected medicinal plant extract as root promoter potential"** submitted by Ms. Trupti Dash (Adm. No. 191221210) to Odisha University of Agriculture and Technology, Bhubaneswar in partial fulfilment of the requirements for the degree of Master of Science in Agriculture (Agricultural Biotechnology) has been approved by the students' Advisory Committee and the external examiner.

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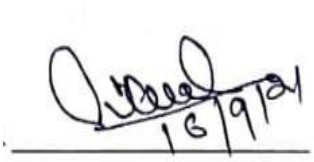

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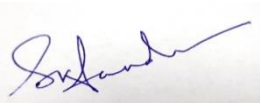

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Bhubaneswar (Trupti Dash)
Date: 13.09.21

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ABBREVIATIONS USED

IAA :	Indol-3- acetic acid
IBA:	Indol-3-butyric acid
TLC:	Thin layer chromatography
HPTLC:	High – performance thin-layer chromatography
FTIR:	Fourier transform infrared
Rf:	Retardation factor
cm:	Centimeter
nm:	Nano meter
µm:	Micro meter
mm:	Milimeter
ml:	Milileter
mg:	Milligram
g:	Gram
hrs/hr:	hours/hour
ppm:	parts per million

ABSTRACT

Medicinal plants are used by Indians for various purposes since along. It has pharmaceutical application in drug making by utilizing its active chemical constituents and also some are consumed directly as food. Mankind being more health conscious and environment responsive are eagerly searching for natural substitute than different synthetic chemicals. Most of the active compounds present in the medicinal plants having various chemical properties including root promoting potentials. These compounds may be compared with known commercial growth regulators biochemically as well as using stem cuttings of ornamental plants. The present study investigated the presence of root promoting chemical constituents derived from the medicinal plant extract of ten different plant species such as *Ocimum sanctum, Azadirachita indica, Nyctanthes, Ayapana triplinervis, Moringa olifera, Plumbago indica, Tinospora cordifolia, Typhonium, Piper betle* and *Mimosa pudica* at 0, 1000ppm, 2000ppm, 3000ppm and 4000ppm concentration. Among the ten different medicinal plant extracts, *Moringa olifera* and *Piper betle* leaves extract showed shoot emergence as well as root induction from cuttings of *Bougainvillea glabra* within two weeks of planting. In control treatments (either without plant extract or growth regulators) did not show any positive response. However, the percentage of rooting is significantly similar with known plant growth regulators (IAA, IBA) with similar concentrations. The best treatments are tested for 24hr and 48 hr duration in three different replications.

Further, biochemical analysis was also performed such as TLC, HPTLC and FTIR analysis to identify the specific chemical constituent present in selected leaf extracts of *Moringa olifera* and *Piper betle*. On the basis of HPTLC analysis, it has been shown that the chromatographic pattern is closely similar with growth regulator like IBA. On the basis of FTIR analysis, it also showed that the plant extracts derived from *Moringa olifera* and *Piper betle* have similar functional groups as compared with IBA and IAA. Further, the plants derived from leave extracts of *Moringa olifera* and *Piper betle* were tested different biochemical assay. The chlorophyll and protein content are closely similar with plants derived from cuttings treated with growth regulators. Thus, through multiple analysis and cross match making it was confirmed that leaf extract of *Moringa olifera* and *Piper betle* contains auxin or auxin like substances which can be used as a substitute to synthetic plant hormones.

INTRODUCTION

Medicinal plants have been used from the Vedic era. Traditionally, medicinal plant sector has obtained a significant position in socio cultural life along with health, nutrition and medicinal requirement of tribal and rural population. As estimated by World Health Organization (WHO) traditional medicines and different plant originated drugs are used by almost 80% population of developing countries, for their primary health care needs. Realizing these importance Government of India established specially designated Department for Indian System of Medicine and Homoeopathy, and National Medicinal Plants Board for further regulation, promotion and maximizing its benefits along with ensuring sustainable growth. Medicinal plant sector has been identified as an area having higher potential by different Ministries and for its conservation, augmentation and commercialization under various programs. Medicinal plants are used by pharmaceutical industries for extraction of high value secondary metabolite and thus getting higher importance. (Rout *et al.,* 2000; Rout and Das, 1997). The therapeutic potential of plant products can be traced back to over five thousand years ago as there is evidence of its use in the treatment of diseases and for revitalizing body systems in Indian, Chinese and Roman civilizations (Mahesh *et al.,* 2008). In India, approximately 7000-8000 plants have been used as therapeutic potential. Medicinal plants have also been used as folk medicines in different indigenous systems and other officially recognized system of medicine like Siddha, Ayurveda, Unani and Homeopathy (Srinivasan *et al.,* 2007). A long history of human interaction with its nature can be recognized by use of herbal medicines. The 16th century B.C. manuscript 'EBER PAPYRUS' recorded the use of castor oil, aloe vera, poppy plant etc. as medicinal herbs. Evidences from Indian Vedic literature such as 'Athervaveda', 'Rigveda', and other literary works of 'Charaka' and 'Susruta' signifies the use of different herbs having medicinal properties. Even ancient European, Egyptian and Greek literature specifies the use of herbal medicines.

India with 2.4 percent of total geographical area of the world, constitute about 8 percent of total biodiversity. Also, India is a treasure house of biodiversity with a large variety of plants as also identified as one of the 'Vavilorian' centers of origin and crop diversity. As per estimation about 49000 species of plants are present of which 5000 are endemic (Kumar and Asija, 2000). About 90 percent of country's biodiversity are concentrated in the mountain ranges of the Himalayas, the Vindhya and Satpura ranges,

the north eastern mountains of Khasi and Mizo hills, the Western and Eastern ghats and northern plateau and peninsular. These areas along with coastal tribal rich areas are the epicenter of traditional medical practices. India medical systems are one of the oldest and adopted by many tribes during their visit and invasion to India. Ancient system such as Ayurveda, Yoga, Unani, Siddha, Homeopathy mostly utilizes herbal plants for their treatment and therapy. Researchers also state that a lion's share of known valuable medicinal plants mostly found in dry and moist deciduous vegetation area as compared to evergreen and temperate forests. When go for classification on the basis of habitat, it was found 33% are trees, 32% are herbs, 20% are shrubs, 12% are creepers and rest 3% are others. Thus, majority of medicinal plants are well developed higher flowering plants.

Plants contains various chemical compounds and metabolites which can be used for the treatment of chronic and infectious diseases. A vast knowledge of medicinal significance of plants against different illnesses thought to be concentrated in areas and locations where it is still used is greater potential. The reason for these properties of plants is its specific chemical constituent which produces a definite physio chemical change in our body. These bioactive compounds are various alkaloids, flavonoids, tannins and phenolic compounds (Eapen *et al.*, 2001). Tribal and rural communities used plant resources for various purposes like food, forage, medicine, household implements, mates, in construction work and fuel purpose.

In past few decades, man being more health-conscious in traditional herbal medicines are again gaining their importance with lesser side effects. In many developing and under developed countries a large portion of rural and tribal poor population relies on medicinal plants for their primary health care needs. Even if modern medicines are available in these areas but still due to cultural and historical reasons these herbal medicines have greater popularity. Throughout the phages of evolution, human beings have been duly recognized the potential of biodiversity encompassing numerous life forms and providing the basic needs of life such as food, fuel woods, fodder, medicines, clothing and shelter, etc. India a country with rich biodiversity having two mega diversity biological hotspots located in Western Ghats and Eastern Himalayas contains many endemic species of higher plants, herbs and shrubs.

Table 1. Global overview of medicinal plant use

Country	Use of tradition medicine	Extent of regulation
China	Extensively used in traditional medicine	Authorized used in national health care
India	Ayurveda, Siddha, Unani, Homeopathy, Yoga	Authorized under central medicine
Canada	Naturopathy, acupuncture, traditional natural north American medicine	No formal recognition or regulation
USA	Hypnosis, naturopathy, biofield therapy	Highly controlled, regulated, licensed and professionally practiced
Japan	Judo therapy, massage/finger pressure, kampo medicine	Restricted used by allopathic physicians
Pakistan	Tibb, Unani, homeopathy	Under national health care system
UK	Osteopathy, hypnotherapy, aquapuncture, spiritual healing	Officially recognized and restricted used by allopathic doctors
South west Asia	Siddha, Unani, spiritual healing, aquapuncture	Integrated in curative and nursing care
Australia	Osteopathy, naturopathy, chiropractic, homeopathy	Restricted and regulated use
Africa	Traditional forms of healing, chiropractic,	Few countries recognized such as Ethiopia, congo, Zimbabwe etc.
Germany	Procaine injection theory, chiropractic, herbal medicine, cell therapy, ozone and oxygen therapy	Used only on unavailability of allopathic treatment
Italy	Prana therapy, anthroposophic and acupuncyure, chiropractic therapy	Chiropractic as a profession, restricted licensed use
Soudi Arabia	Health food products and herbal, nutritional, homeopathy	Used only after registration
Korea	Moxibustion, oriental medicine, chiropractics, acupuncture	Under insurance covered

Along with primary health care these medicinal plants are used for treatment of chronic diseases like cancer, heart diseases, hepatic and other old age diseases like osteoporosis, memory loss and diabetes. More than 90,000 species of plants are used by human being throughout the world. Of that about 90% of medicinal plants are propagated and collected from wild by various industries in India. So there is a greater need to recognize these hidden potential of these mega diversity which can be conserved and utilized to a larger extent. Maximum medicinal plants are distributed in 2000-2500 m altitude and decrease with increasing height. In Himalayan region most of these are found of which about 170 plants are native and 95 are endemic. The industries related to medicinal plants are growing at a rate of 10-15% annually, hence need to make sustainable use with conservation. As per some estimation, in India the value of medicinal plant related trade will increase to the turn of about Rs 5000 crores per annum while at USA, about 5 trillion by the year 2050. So, in this case medicinal and aromatic plants can play an important role in the subsistence livelihood enhancement of rural people, especially women in an environmentally sustainable manner (Sharma *et al.*,2017). Some medicinal plants also utilized as pleasant condiments, flavor, dye, conserve food etc. Phytochemicals have been used as anti-oxidation, anti-allergic, antibiotic, hypoglycaemic and anti-carcinogenic. These excessive extraction and commercialization of these industry without proper regulation has resulted in destruction of biodiversity. With further extensive research and development it is exploring more potential use in other sectors like as a growth hormone substitute, as alternative to other chemical compounds and many more. With this, there are growing interests in using natural antimicrobial compounds, especially extracted from plants, for using it as a bioactive compound which can promote rooting.

Ornamental plants are very essential in environmental beautification and management, they make public parks and houses more conducive for relaxation and enjoyment (Day and Loveys,1998). Among ornamental plants, flowering plants are associated with mankind since the dawn of civilization. These are one of the wonderful creations of nature. In general, most of the perennial ornamental plant species are multiplied and propagated by the use of various propagation techniques such as cuttings, layering or grafting (Xiong *et al.*, 2000). The use of cuttings from stems, leaves, roots or terminal buds are considered the most common practice due to its practicability and easy application especially in developing countries. Internal and

external factors are largely associated with the process of regeneration so also the type of wood selected (Farooqi, 1994). Cutting is a well-known common horticultural practice in the propagation of many ornamental, medicinal and fruit crop species. Induction of adventitious roots from cuttings is governed by the complex interaction of several factors including growth hormone interaction, enzymatic activities and physiological status of stem cutting are important factors to achieve the maximum percentage of root induction. Plant growth regulators are synthetic chemical compounds which applied to the plants to promote growth and development of plants. They used to stimulate root development in stem cuttings. In the history of plant propagation method, the treatment of cutting with growth hormone for root initiation was a major milestone. Apart from all growth regulators, auxins are most readily used for root induction of which Indole acetic acid (IAA), Indole butyric acid (IBA) and Naphthalene acetic acid (NAA) are used either in liquid or in powder form for treatment of stem cuttings. This treatment enhances the growth by increasing the percentage of cuttings which forms roots, hastens root initiation and enhance root numbers. This chemical treatment resulted in enhancement of the number of adventitious roots that are naturally able to regenerate into roots; however, their effectiveness varies among different species and varieties.

Keeping in view the above facts, present investigation entitled “Evaluation of bioactive compounds from selected medicinal plant extract as root promoter potential” was attempted with the following major objectives,

Objective,

1) To evaluate the active ingredients content in the selected medicinal plants.

2) To evaluate the root promoting activities by using the plant extract.

REVIEW OF LITERATURE

India is reach heritage of plants, shrubs and herbs. Indians have wide knowledge of plant originated drugs from many decades. It is also one of the most suitable and conducive environmental condition for the development of rich diversity of medicinal plants due to its geographical position, wide variations in soil and subtropical climatic condition. The sub-continent is suitable for cultivation of large number of medicinal and aromatic plants which can be used as raw materials for pharmaceutical, cosmetics, perfumery, flavor and agrochemical industry. As per the report of WHO, about 4 billion people (80% of the world population) use herbal medicines for some aspect of primary health.

2.1 MEDICINAL PLANTS AND ITS ANTIMICROBIAL PROPERTIES

The medicinal effect of plants is the result of presence of secondary metabolites. These are not the effect of single compound but a combination of many metabolites. These can be extracted and can be used as drugs in place of synthetic chemicals to solve the problem of antibiotic resistance, hypersensitivity, immune suppression and different allergic reaction. Ali Rehman *et al.,* (2002) proposed the antimicrobial activity of *Azadirachita indica* against *Microsporum canis, Aspergillus fumigatus, Candida albicans*, etc. The aqueous and ethanolic extract of leaves and roots have higher inhibition effect. Uma and Sasikumar (2005) carried out that organic and alcoholic extract of *Calotropis gigantia, Moringa olifera, Justica adhatoda* and *Piper betle* contains antimicrobial, antifungal, antibacterial activity against *Escherichia coli, Bacillus subtilis, Aspergillus niger* and *Rhizopus sp*. According to Kharate and Tambekatr (2005) the leaves extract of *Ocimum sanctum* have antimicrobial activity against Escherichia *coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomanas aeruginosa* and *Enterococcus faecalis*. Also, the leaves and whole plant extract of pudina and beetle showed similar activity.

Balakrisahna *et al.,* (2006) tested the antimicrobial performance of *Mimosa pudica, Sida cordifolia* and *Angle marmelos* against *Pseudomanas aeruginosa*, against *Escherichia coli, Salmonella typhi, Aspergillus niger* and *Rhizopus sp*.

2.2 CHROMATOGRAPHIC TECHNIQUES

Chromatographic techniques are the most common technique extensively used for different biochemical analysis like separation, isolation and purification of metabolites and drugs. Thus, these are used as useful tools for derivation of preliminary information about the physicochemical properties of the metabolites in comparison to the drugs. Though this analysis gives little information about specific chemical structure, but by comparing the standard and different analytes through their chromatographic properties authentic conclusion can be derived to establish the identity of a particular metabolic product. HPTLC is an improved method of TLC which uses the conventional technique of TLC in more optimized way. It is also known as planar chromatography or Flatbed chromatography.

2.2.1 TLC AND HPTLC

Thin layer chromatography (TLC) is most widely used relatively easy to use, cheep, rapid and robust. These are used prior to spectroscopic techniques for metabolic studies in analysis for isolation and purification of analytes. This has widespread use with diverse variety of stationary phases like silica gel, alumina and a number of bonded hydrocarbon phases used for either ion exchange or reverse-phase separation. Generally, these phases are coated on glass support, plastic or aluminium foil support. With recent advancement new improved and higher efficient TLC stationary phase techniques are evolved with the introduction of HPTLC plate i.e. higher performance thin layer chromatographic plates coated with a layer of 5 µm particle size silica which are more advantageous in terms of speed of chromatographic development and resolution. Another type of chiral TLC phase is also their showing higher performance, however their use is very limited in studies of biological analysis. These chromatographic phases are developed when ultraviolet absorbing analytes are exposed in fluorescent indicators for detection. In alternative way these can also be visualized by spraying wide range of chromatographic reagent. Various class of metabolites are observed through colour reaction when provided with appropriate assistance for example p-dimethyl amino benzaldehyde used for detection of glycine conjugates which produce characteristic red orange colours, glucuronides yield characteristic colour on treatment with napththa resorcinolis, potassium dichromate silver nitrate for sulphur (II) and ninhydrin for glutathione conjugates. Isolation of metabolites, for

subsequent identification by spectroscopic techniques is the main application of TLC. The inorganic ions are separated through starch binder to provide some firmness to the layer and described as surface chromatography. The use of TLC/HPTLC now expanded considerably with the identification of stationary and mobile phase and deployment of forced flow (FF) and gradient flow TLC method (Poole and Poole,1994., Sherma,1994).

2.3 PLANT EXTRACT AS GROWTH HORMONE SUBSTITUTE

Plant hormones and growth regulators are used by human being for many decades in tissue culture and agriculture. With the enhancement of scale of application, there originate the risk of toxicity in plants and animal which leads towards the search for natural compounds for replacing the synthetic hormones (Culter *et al.*,1990). Several studies have shown that plant extracts have an effect on the vegetative growth of many plants. It is an alternative of natural compounds which can be used as phytohormones (Wiesman and Jaenicke,2002). Stirk and Staden (1996) reported that six commercially used seaweed have positive result for cytokinin and auxin like substances. They conducted soybean callus bioassay and mung bean rooting bioassay followed by co-chromatographic analysis which showed zeatin and zeatin riboside like substances, improving rooting in mung bean. Awad *et al.,* (2017) reported that the leaf extract of *Moringa olifera* have been used for evaluation of their effects on rooting of cereal forages (*Sorghum bicolor* L, *Penisetum typholium* and *Sorghum sudanes*). They derived that higher concentration of extract produces higher growth and forage yields as compared to others. Fadia El Sherif (2017) studied aloe vera extract as a cost effective and environment friendly substitute to synthetic plant growth regulators and phytohormones. The study identified the promotory activity of aloe vera leaf extract in increasing plant height and weight, roots and leaves, number of shoots, the root length and mineral concentration of *Populus* clones. Hekmat Y. A. Massoud *et al.,* (2017) experimented that the highest value of vegetative growth of rosemary were obtained when the stem cuttings are treated with coconut milkat the highest rate (75%). They also concluded that natural product such as coconut milk, yeast extract, seaweed extract and honey were much better than chemical growth regulators like IBA, IAA.

Gad and lbrahim (2018) reported that the rooting has been achieved from Picual olive cutting by some natural extracts containing auxin such as garlic (20%), liquorice

(10 g/l), Moringa (20%), Yeast (10%) and Algae (5 cm/l) when compared with IBA 4000 ppm. Also stated that Moringa extract resulted higher performance in comparison to other natural extract. Ibironke (2019) reported that the Indole -3-butyric acid and the coconut water had significant effect on the root emergence and root growth of *Bougainvillea* species compared to the other synthetic hormones used. Al-Habib *et al.,* (2019) studied the effect of onion root exudate, date fruit aqueous extract and yeast as root promoter on the stem cutting of *Quisqualis indica* shrub. Their result indicate that highest concentration and longest dipping period gave the best result, however, among all onion root exudate showed highest response with respect to percentage of rooting, root number and root length as compared to the standard taken IBA (1000 mg/l). Abdul *et al.,* (2020) reported that some of the medicinal plants are an important source of natural bioactive compounds which can promote rooting, of semi hard wood cuttings of Olive, *Punica*, Myrtus and *Ficus*. They investigated the effect of a number of natural products like Garlic, Black cumin seeds, Liquor ice roost and Egyptian cassia flowers as synthetic growth hormone substitute. They derive that treating the stem cuttings in the extract (2.5 and 1.25 gm/l) for 48 hrs treatments produces comparable result as application of IBA 1000 mg/l. Bahed and Al-Habib (2020) conducted an experiment to evaluate the activity of *Petroselium crispum* aqueous extract as a substitution of IBA. They had taken aqueous extract of parsley in varied concentration (1.25, 2.5 g/l), compared to IBA (100 mg/l). The stem cutting of *Rosmarinus officinalis*, *Nerium oleander*, *Olea europaea*, *Plumeria alba*, *Hibiscus rosa sinensis*, *Pelargonium graveolens* were dipped in the treatment foe 24 hrs. Observation were taken 30 days after planting such as rooting percentage, number and length of roots, number of new leaves and branches. They found significant difference between treatment and concluded that parsley extract (2.5 g/l) shows better result compared to other treatment. Further biochemical analysis of parsley extract shows the presence of IAA, GA_3 and cytokinin etc. Bahedh and Habib (2020) studied the utilization of crude plant extract of different plant species such as Parsley (*Petroselinum crispam*), Dill (*Anethum graveolens*) and date palm fruits (*Phoenix dactylifera*) for induction of rooting from apical stem cutting of rosemary plant in comparison to growth hormone IBA. They concluded that the Parsley extract in the concentration (2.5 g/l) showed significant result.

2.4 EFFECT OF APPLICATION OF GROWTH HORMONE ON STEM CUTTINGS

Propagation of ornamental plants through cuttings is the cheapest and easiest method. Stimulation of root development from the stem cuttings was carried out by application of synthetic growth regulators. Singh (2000) observed maximum sprouting percentage, rooting percentage, number of shoots per cutting, number of leaves per cutting, optimum shoot length and plant establishment in Bougainvillea peruviana cv. Shubra when treated with IBA 500 ppm, irrespective of duration (12 and 24 h) of treatment and method of planting. Singh and Singh (2002) studied the effect of growth hormones (IBA and NAA) and type of wood on rooting behaviour of *Bougainvillea peruviana* cv. Thimma. They found remarkable increase in rooting percentage, number of sprouts per cuttings, number of leaves per cuttings, length of sprout, diameter of sprout, number of roots per cuttings, length of root, diameter of root, fresh weight of roots per cutting in hardwood cutting with application of IBA 2000 ppm. Also, it resulted in early sprouting and maximum percentage of rooting whereas no differences were reported with respect to different types of wood on number of days taken to sprouting. They also reported a significant Interactions between type of wood and growth regulators concentration with maximum result when treated with IBA (2000 ppm). Singh (2002) reported the effect of growth hormone (IBA and NAA) application on sub terminal cuttings of *Bougainvillea* cv. Thimma. It was found that higher rooting percentage was obtained when treated with IBA (4000 ppm). Also, it was realized to be one of the easiest and most convenient method for propagation of Thimma variety of *Bougainvillea* rather than traditional method of air-layering. Adiga *et al.*, (2004) observed higher mean percentage of rooting in hardwood cutting of Bougainvillea when treated with NAA (1000 ppm). Whereas, the highest average number of roots in Hibiscus was observed with NAA (1000 ppm). Similarly, highest rooting in *Jasminum multiflorum* cutting was obtained with treated with IBA (500 ppm). Khan *et al.,* (2005) reported higher number of roots and rooting percentage in cutting of Damask rose with the increase in the hormone application. They also observed that NAA (50 mg/l) resulted in maximum number of roots and percentage of rooting as compared to control. Gupta *et al.,* (2005) observed maximum root length in *Bougainvillea* cv. Pallavi semi hardwood cutting when treated with IBA 4000 ppm as compared to other different concentration. Thus, IBA 4000 ppm showed effective and maximum

vegetative propagation in semi-hardwood cuttings of cvs. Pallavi and Mahara variegate. Thorat *et al.,* (2006) reported the maximum sprouting, rooting and survival rate of plant in Nerium cutting when treated with IBA (100 ppm) for 24 h. Lima *et al.,* (2006) studied the treatment of different concentration of NAA solutions (0, 1, 500 and 3,000 mg/l) for 10 sec. along with different growing media such as carbonized rice hull and vermiculite for rooting of semi-hardwood cuttings in *Calliandra selloi* (rose and white stamens) and *C. tweediei* (red stamens). They found that *C. selloi rose*, *C.selloi* white and *C. tweediei* red showed better percentage of rooting in carbonized hull media. The experiment also confirmed zero interaction between specific growth medium and varied NAA concentrations without any significant difference. Hashemabadi and Sedaghathoor (2007) found that treating Camellia japonica cuttings with various concentration of growth hormones such as IBA (4000 mg/l), IBA (4000 mg/l + NAA 2000 mg/l) and IBA (4000 mg/l) produced maximum number of roots, higher root length and highest percentage of rooting respectively. Parvez *et al.,* (2007) experimented the effect of growth hormones and planting times on Peach cutting. For which individual stem cuttings were treated with various concentration of IBA (0, 500, 1000, 1500 and 2000 ppm). However, they found increased growth parameters irrespective of planting time. Stem cuttings treated with 2000 ppm IBA produced the highest number of branches per plant, number of roots per plants, root length, root weight, and plant survival rate. Bhatt *et al.,* (2008) experimented with the cutting of *Lavendula* and found maximum callusing, very early root growth, higher percentage of rooting along with root numbers and root length when treated with IBA. Hussein (2008) reported the seasonal effect on plant establishment from semi-hardwood stem cuttings of *Thunbergia grandiflora*. For that the cutting were planted with two months interval. It was observed that the cutting planted on March 21 showed remarkably increased growth in percentage of rooting, higher numbers of roots per plant along with increased leaf numbers, dry and fresh weight of leaves and dry weight of branches per as comparison to others. Kumar *et al.,* (2008) reported the effect of growth hormone (IBA and NAA) on *Thunbergia grandiflora* for rooting from stem cutting. They applied both the hormones individually and in combination as IBA, NAA, IBA+NAA at different concentration (0, 1000, 1500 and 2000 ppm). Among all combined application of IBA +NAA produced maximum rooting with good establishment. Laubscher and Ndakidemi (2008) reported the influence of IAA applied in varied concentrations (0, 500, 1000, 2000 and 4000 ppm) on rooting of *Leucedendron laxum* in a shade tunnel

house. For this experiment different growth mediums are taken such as combination of bark +polystyrene (1:1), peat moss +polystyrene (1:1), bark + river sand +polystyrene (1:1:1) and perleite + river sand (1:1). The result indicated the superior behaviour of different growth aspects with bark + polystyrene and peat + polystyrene mediums with application of auxin as comparison to others. Also, it signifies shade tunnel as one of the most effective and favourable environmental condition for plant establishment from cuttings. Reddy *et al.,* (2008) studied the effect of different concentration of growth hormone in *Ficus carica* cuttings. treated with IBA (2500 ppm) + NAA (2500 ppm) They found higher rooting percentage, root numbers, root length and survival rate of cuttings when treated with combined application of IBA (2500 ppm) and NAA (2500 ppm) after that IBA (2500 ppm) produce significant result in both semi hardwood and hardwood cuttings. When NAA (5000 ppm) was treated, it was observed that hardwood cuttings resulted in the maximum rooting percentage, higher survival rate and other rooting characteristic in comparison to that of semi hardwood cuttings. Parmar *et al.*, (2010) experimented the effect of various concentration of IBA on rooting behavior of *Bougainvillea peruviana* cv. Torch Glory hardwood cuttings. For number of days of treatment IBA (4000 ppm) was best and for better root and shoot characters and survival combined application of IBA (2000 ppm) +NAA (2000 ppm) were better. Karami and Salehi (2010) reported the effect of growth hormone (IBA and NAA) on rooting of cutting in *Tecomella undulata*. They found maximum percentage of rooting from hardwood and semi hardwood cutting when treated with NAA, however, maximum rooting of semihard wood cuttings and hardwood cuttings was reported when treated with IBA.

Singh *et al.,* (2011) studied the effect of different concentration of IBA on stem cutting of *Bougainvillea glabra* var. Torch Glory. He observed that IBA (2000, 2500 and 3000 ppm) showed highest rooting and sprouted of cutting whereas IBA (3000 ppm) showed higher length of sprout per cutting and maximum number of rooting per cutting. Singh and Singh (2011) reported the differential rooting behaviour of *Bougainvillea* from hardwood cuttings of various varieties i.e. Mrs. Butt, Louise Wathen, Shubra and Thimma. They found significant influence of IBA concentration and varietal difference on rooting, sprouting, callusing and cuttings establishment, however among all varieties experimented, only Lousie Wathen cuttings showed maximum percentage of rooting, shooting and callusing and 100% plant establishment

when treated with 1000 ppm IBA. Ogunwa (2011) analyzed the effect of coconut water as root stimulation in cutting of *Bougainvillea spectabilis* and *Bougainvillea glabra*. He treated the stem cutting for different duration as short duration (5 mins-10 mins), medium duration (1 -3 hrs), long duration (6 -10hrs) and no dipping as control. The cutting dipped for 1 hr showed best performance. Kako (2012) observed the variable relationship between IBA application and the cutting diameter of hardwood cuttings of black Mulberry. Remarkable effects were obtained such as higher rooting percentage, shooting percentage, length and diameter of transplant establishment with varied doses of IBA (0, 1000, 2000, 3000 and 4000 mg/l). Successful budding of scion was observed with combined application of IBA and kinetin. Treatment of 12-14mm hardwood cutting with 4000 ppm IBA produced better rooting and 10-11mm hardwood with 2000 ppm IBA produced better establishment. Abu-Zahra *et al*, (2012) conducted an experiment analysing the effect of NAA on *Partheno cissus* plant, one of the most attractive outdoor deciduous vines. They had taken 6 different concentrations of NAA (0, 1000, 2000, 3000, 4000 and 5000 ppm) for treatment of cuttings. Among all 1000 ppm NAA produced significant result with better growth. Adekola and Akpan (2012) studied the effect of growth hormone on the stem cutting of *Jatropha curcas* but got minimal effect on survival and sprouting in comparison to untreated cuttings taken as control. However, remarkable effect on rooting behaviours were observed where IBA treated cutting produced better root than those of NAA treated. Asl *et al.,* (2012) reported highest number of rooting from semi -hardwood cutting of *Bougainvillea* with treatment of IBA 2000 ppm for 7 second. It also resulted in higher root length and number of roots in comparison to control. Rahbin *et al.,* (2012) observed the rooting characteristic of cutting of 'Night Jasmin' taking IBA conc. and the effect of location of shoot as variability. For the experiment the cutting were treated with IBA (0, 1000, 1500, 2000 and 4000 ppm) for 5 seconds and taking pot mixture containing sand and peat moss in mist greenhouse condition. IBA (2000 and 4000 ppm) produced better result in comparison to other treatments. Singh (2012) reported the effect of different concentration of IBA on stem cutting of *Bougainvillea* var. Louise Wathen, Thimma, Mrs. Butt and Shubra. The highest number of sprouting, rooting, callusing and establishment of cutting was observed with IBA 1000 ppm. Alshammary *et al.,* (2013) studied the effect of growth regulators in *Hamelia patens* and *Bougainvillea* cv. Shubra. They observed the maximum rooting percentage, bud breaking per cutting, number of roots per cutting, root length and establishment percentage along with higher number of

branches per cutting when treated with 2000 ppm IBA. Whereas considerable variation found when hardwood cuttings were treated. Abu-zahra *et al.,* (2013) analyzed the rooting behavior from the cutting of Rosemary, Hedera, *Syngonium* and *Gardenia*. He found the maximum percentage of rooting in rosemary and hedera when treated with NAA (3000 ppm), however highest in *Syngonium* and *Gardenia* when treated with (NAA 1000 ppm and 4000 ppm). Hassanein (2013) studied factors associated with efficient plant propagation from steam cutting. Various factors considered by him were type of cutting taken, medium of planting, and different concentration of growth medium. For that he had taken *Ficus hawaii* and *Chrysanthemumm orifolium* and observed the rooting behavior. Among all sand media and peat moss showed maximum survival and rooting. Peat moss +50 ppm of IAA was efficient for propagation of *Ficus* where as a mixture 1:1:1 of peat moss, sand and perlite + 50 ppm of IAA was effective for *Chrysanthemum.* Memon *et al.*, (2013) observed the significant effect of different concentration of NAA on sprouting and rooting of stem cutting in *Bougainvillea* irrespective of varietal difference. They found optimum growth with highest sprouts per cutting, number of sprouting, length of sprouts, number of roots, length of roots, roots per cutting and leaves per cutting when treated with 6000 ppm NAA.

Kumari *et al.,* (2013) studied the rooting characteristic of *Jatropha curcas* and found the highest number of shoots per cutting, early emergence of shoots, optimum number of sprouting and shoot length when treated with IBA 200 ppm. Seyedi *et al.,* (2013) analyzed the rooting of *Bougainvillea glabra* when different type of stem cutting was treated different concentration of growth hormones. The treatment containing IBA (4000mg/l) resulted highest rooting. Also found that semi hardwood cuttings of *Bougainvillea glabra* were the best for propagation. Sahariya *et al.,* (2013) analyzed the rooting behavior of *Bougainvillea* var. Thimma cutting in various external condition such as in open field and polyhouse along with treatment of various concentration of IBA. He obtained significant result under polyhouse condition as compared to open field with more pronounced effect with increasing concentration of IBA (1500-2000 ppm). However, highest number of root cuttings, percentage of rooting, length of root per cutting, length of shoot per cutting and dry weight of both root and shoot was resulted from IBA 2000 ppm. Susila and Reddy (2013) obtained the highest rooting along with longest root length in *Adathoda vasica* when treated with IBA 1500 ppm, whereas maximum roots per cutting and fresh and dry weight of root

was obtained with NAA 1500 ppm. Shabha and Alshammary (2013) studied that the highest rooting percentage, many number of roots per cutting and maximum establishment percentage in *Bougainvillea* cv. Shubra cutting was obtained when treated in IBA 500 ppm, soaking for 12 or 24 hours. Ullah *et al*., (2013) reported that the effect of auxin concentration (NAA and IBA) on regeneration of Marigold. They observed IBA 100 ppm resulted in maximum leaves per plant, maximum plant height and increased root size. However, IBA 200 ppm produced maximum leaves per plant and maximum branches per plant. Also, maximum effect on root growth obtained in IBA 400 ppm, after that increase in the IBA concentration had retarded effect on root size. Likewise, in case of NAA application proportional effect was observed in the growth of leaves per plant with increase in NAA concentration. Maximum plant height, maximum increase in roots per plant and root size was obtained at 100, 200, 300 ppm NAA respectively. Yan *et al.,* (2014) reported that the adventitious root development from the stem cutting of *Hemarthria compressa* was stimulated by the application of auxins which increase the number and length of root formation. Tripathi *et al.,* (2003) used various growth promoting substances (NAA, IAA, IBA,2,4,5-T) for induction of rooting and rooting quality of *Poinsettia*. They also observed that the highest rooting was observed in IAA treated cuttings (97.78%) while the highest number of roots per cutting was observed in 1000 ppm NAA. The longest root was observed in 200 ppm IAA, whereas, the highest root fresh weight was recorded with 100 ppm IAA (2.30 g). Akhtar *et al*., (2015) studied the rooting performance of *Rosa centiflora* cutting with application of different growth regulators. There were total 31 different treatments of growth hormones (IBA, IAA, NAA) along with BAP for root induction. IBA (450 ppm, 700 ppm and 950 ppm) produced optimum result in comparison to others. However maximum shoot length, shoot dry weight, root length and root dry weight was observed in IBA,450 ppm. They also found that plant performs better in lower concentration of growth regulator as compared to higher concentration.

Renuka and Sekhar (2014) reported the rooting characteristic of cutting of carnation (*Dianthus caryophyllus* L.) at different concentration of growth hormones. Two concentration of treatment IBA 200 ppm and IBA 100 ppm + NAA 50 ppm was taken for analysis of which the former resulted in higher number of rooting, maximum percentage of rooting, highest number of roots per cutting and maximum percentage of establishment of cutting than the latter. Singh *et al.,* (2014) reported that effect of

growth regulator on the rooting behavior in *Morus alba* stem cutting. They adopted quick deep method to treat the stem cutting in various concentration (1000, 1500 and 2000 ppm) of growth regulators. The treatment with 1000 ppm IBA recorded maximum percentage of sprouted cutting, higher roots/cutting length. Rahdari *et al.*, (2014) observed the highest root length, number of branches, dry and fresh weight of root from the stem cutting of *Cordyline terminalis* when treated with combination of NAA (2000mg/l) +IBA (1000 mg/l) as compared to other combination. Wazir (2014) reported the performance of rooting from Camellia cutting with varied concentration of growth hormones. For which he had taken different types of cutting such as soft wood, semi hard wood and hard wood, which were treated in various concentration of growth hormones (250 -500 ppm NAA and 500 -1000 ppm IBA) for few minutes before potting. From those only 1000 ppm IBA showed effective rooting irrespective of different types of cutting.

MATERIALS AND METHODS

The present study was carried out at Department of Agricultural Biotechnology, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha. The detailed materials methods and the experimental techniques are presented below.

3.1 Selection of medicinal plant species:

Medicinal plant species such as *Ocimum sanctum*, *Azadirachita indica*, *Nyctanthes arbor-tristis*, *Ayapana triplinervis*, *Moringa oleifera*, *Plumbago* zeylanica, *Tinospora cordifolia*, *Typhonium trilobatum*, *Piper betle* and *Mimosa pudica* were selected for the experiment (Table 2).

Table.2. Different medicinal plants and their active principle used for the experiment.

NAME OF THE PLANT	PHENOTYPIC CHARACTER	MEDICINAL USE	ACTIVE PRINCIPLE	REFERENCE
Nyctanthes arbor-tristis	Shrub	antibacterial, antihelmintic, anti-inflammatory, anti-pyretic, antioxidant, antifungal and hepatoprotective activity	nicotiflorin, nyctanthic, oleanolic acid, bita-sitosterole, astragaline	Mahida Y *et al.*,2007, Saxsena RS *et al.*,1984, Rathee JS *et al.*,2007.
Moringa oleifera	Deciduous tree	stimulant, antispasmodic, expectorant and diuretic, cardiac tonic, antiepileptic, nervous debility, asthma	Glucosinolates, isothiocyanates, niazimicin, pterygospermin, benzyl isothiocyanate	Fahey *et al.*,2001, Jaiswal *et al.*,2009, Abigos *et al.*,1999
Piper betle	Vine	wide variety of disorders such as skin diseases, rheumatism, inflammation, syphilis, mental illness, epilepsy, hysteria, dehydration, and diarrhea	Piperol-A, Piperol-B, methyl piper betlol	Verma S *et al.*,2010, Chopra RN *et al.*,1956
Azadirachita indica	Deciduous tree	immunomodulatory, antihyperglycaemic,	Nimbidin, Nimbin, Sodium nimbidiate, Gedunin,	Biswas *et al.*,2002, David and S.N., 1969

		antiinflammatory, antifungal, antiulcer, antimalarial, antiviral, antibacterial, antifungal, antioxidant, anticarcinogenic and antimutagenic properties	Mahmoodin and azadirachtin	
Mimosa pudica	Creeper	treatment of leprosy, leukoderma, blood diseases, biliousness, diarrhoea, dysentery, insomnia, inflammations and burning sensation	mimosine, mimosinamine, mimosinic acid, tyrosine 3, 4-dihydroxypiridine	Nair LS *et al.*,2007, Kirk LF *et al.*,2003
Ayapana triplinervis	Perennial Herb	antiseptic, haemostatic, antibacterial and anti fungal activity	ayapanin and ayapin, stigmasterol, esculetin methylene ether	Anne GF *et al.*,2008
Plumbago zeylanica	Herbaceous Shrub	Antibacterial, also active against certain yeasts and fungi (Candida, Trichophyton, Epidermophyton and Microsporum spp.) and protozoa (Leishmania)	Plumbagin(5-hydroxy-2-methyl-1, 4-naphthoquinone-C11H8O3	Vishnukanta *et al*.,2010, Mandavkar YD *et al*.,2011, Vishnukanta *et al*.,2010
Tinospora cordifolia	Herbaceous vine	anti-neoplastic and anti-oxidant, anticancer, neurological immunomodulatory, vasorelaxant, psychiatric activities	**Berberine,** 18-norclerodane glucoside,Palmatine, Cordifolisides A to E	Nadkarni *et al*.,2010, Sharma A *et al.*,2010, Rout G.R.,2006
Typhoniumtrilobatum	Herb	anti-inflammatory, anti-bacterial, anti-fungal and anti-diabetic	beta-sitosterol, anti-nutritionals like phytate and oxalate	Banerjee *et al.*,2015, Shaf F *et al.*,2005
Ocimum sanctum	Shrub	healing properties, anti-cancerous, Antioxidant, Antidiuretic	Eugenol	Singh *et al.*,2007, Naquvi *et al*., 2012

3.1.1 TULSI - *Ocimum sanctum* L (Carl Linnaeus)

Taxonomic hierarchy

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Lamiales
Family: Lamiaceae
Genus: Ocimum
Species: sanctum
Subfamily: Nepetoideae
Tribe : Ocimeae
Genus: *Ocimum*
Species: *sanctum*

Basil has been used for thousands of years in Ayurveda having healing properties, whose whole plant is useful including leaves, seeds, stem and roots, containing a variety of chemical constituents. Eugenol is the active constituent responsible for various therapeutic potential (Singh *et al*.,2007), along with it tulsi oil also contents b-bisabolene (13-20%), methyl chavicol (3-19%), 1, 8-cineole (9-33%), eugenol (4-9%), (E)-a-bisabolene (4-7%) and a-terpineol (1.7-7%) (Naquvi *et al*., 2012). The alcoholic extract of leaves of ocimum contains enzymes likecytochrome P450 cytochrom b5, aryl hydro carbon, hydroxylase and glutathione s-transferase (GST) which are having detoxification effect on carcinogens and mutagens by influencing carcenogenic metabolizing enzyme (Vani *et al*.,2009). It is found throughout the tropical and subtropical regions, however best growth measures are obtained in hot and dry climate, welldrained soil with slightly acidic pH, humidity 94% and temperature ranging from minimum 17^0c to maximum 39^0c (Mondal *et al*.,2009).

3.1.2 NEEM - *Azadirachta indica* A.Juss (Adrien-Henri de Jussieu)

Taxonomic hierarchy

Kingdom: Plantae
Division: Magnoliophyta
Order: Sapindales
Family: Meliaceae
Genus: *Azadirachta*
Species: *indica*

Neem is a tropical evergreen plant native to east india and Burma. It is having many medicinal utilities such as immunomodulatory, antihyperglycaemic, antiinflammatory, antifungal, antiulcer, antimalarial, antiviral, antibacterial, antifungal, antioxidant, anticarcinogenic and antimutagenic properties (Biswas *et al.*,2002). Neem

oil is used as nematicide as well as insecticide as it contains Nimbidin, Nimbin, Sodium nimbidiate, Gedunin, Mahmoodin and azadirachtin (David and S.N., 1969). Neem seed cake is used as soil amendment and also lowers nitrogen losses by inhibiting nitrification. Neem bark and roots are also used to control fleas and sucking pests in powdered form (Khalid *et al*.,1989). Neem seeds are having higher germination percentage and seed planting is the most common way to plant propagation. It is drought resistance and thrive well in areas with an annual rainfall of 400-1200mm. it can grow in many different soil with annual temperature of 21-32^{0}C.

3.1.3 DRUMSTICK - *Moringa oleifera* Lam. (Jean-Baptiste Lamarck)

Taxonomic hierarchy

Kingdom: Plantae
Division: Magnoliophyte
Class: Magnoliopsida
Order: Brassicales
Family: Moringaceae
Genus: *Moringa*
Species: *oleifera*

Moringa plants are naturalized in tropical and subtropical areas and native to Indian sub-continent. It is a deciduous tree or shrub, fast-growing and drought resistance. Traditionally these are used as stimulant, antispasmodic, expectorant and diuretic. Almost every part of plant is of various use. Fresh roots are acrid and vesicant, used as stimulant and antilithic. The bark extract is used as emmenagogue, abortifacient, antibacterial and antifungal activity. Also, flowers are claimed to have be cholagogue, tonic and diuretic activity. The root juice are having medicinal properties and used in cardiac tonic, antiepileptic, nervous debility, asthma and in the treatment of enlarged liver. Seeds contains about 40% nondrying oil known as ben oil used as lubricant in delicate machinery, lubricating watches and in arts. The plant is rich in various simple sugar, rhamnose and other unique compounds like glucosinolates and isothiocyanates (Fahey *et al*.,2001). Other specific active chemical constituents having hypo-tensive, anticancer, and antibacterial activity include 4-(4'-O-acetyl-α-L-rhamnopyranosyloxy) benzyl isothiocy-anate (Abdulkarim *et al*.,2005), 4-(α-L-rhamnopyranosyloxy) benzyl isothiocy-anate (Abigos *et al*.,1999), niazimicin, pterygospermin, benzyl isothiocyanate, and 4-(α-Lrhamnopyranosyloxy) benzyl glucosinolate(Jaiswal *et al*.,2009).

3.1.4 PARIJAT - *Nyctanthes arbortristis* linn

Taxonomic hierarchy

Kingdom:	Plantae
Division:	Angiosperm
Class :	Eudicots
Order :	Lamiales
Family:	Oleaceae
Genus :	*Nyctanthes*
Species:	*arbortristis*

Nyctanthes is native to india and grows naturally in indo Malayan region and Burma. The name nyctanthes states for "night flowering" and specific name arbortristis means "the sad tree" is derived from its dull looks and small tree growing up to 10 m. The leaves contains nicotiflorin, nyctanthic, oleanolic acid, bita-sitosterole, astragaline et., having antibacterial, antihelmintic, anti-inflammatory, anti-pyretic, antioxidant, antifungal and hepatoprotective activity (Mahida Y *et al*.,2007, Saxsena RS *et al*.,1984). Also, flowers are having various active metabolites like nyctanthin and other essential oil with anti-bilious, anti-filarial, sedative and diuretic activity (Rathee JS *et al*.,2007, Alamgir *et al*.,2010). Other plant parts like seeds, bark and stems contains arbortristoside A&B, Glycerides of linoleic oleic, nyctanthic acids, Glycoside-naringenin-4'-0-β-glucapyranosyl-αxylopyranoside and β-sitosterol having Antibacterial, Antifungal, Immunomodulatory, Antileishmanial, Anti-microbial, Antipyretic, Antioxidant activity (Vishwanatara*et al.*,2010).

3.1.5 TOUCH ME NOT - *Mimosa pudica* L.

Taxonomic hierarchy

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Mangnoliopsida
Order:	Fabales
Family:	Fabaceae
Sub family:	Mimosoideae
Genus:	*Mimosa*
Species:	*pudica*

Mimosa is a sensitive plant also called chuemue widely distributed in different parts of india. It is widely known for its rapid movement a peculiar nyctynastic movement also called semimonastic movement. Each part of the plant contains various types of chemical constituents having medicinal properties used in treatment of leprosy, leukoderma, blood diseases, biliousness, diarrhoea, dysentery, insomnia, inflammations

and burning sensation (Kirk LF *et al*.,2003). It contains alkaloids, flavonoids, saponins, tannins and phenolics like mimosine, mimosinamine, mimosinic acid, tyrosine 3, 4-dihydroxypiridine(Nair *et al*.,2007). It also contains a new class of phytohormones – turgorines which shows the ability to bind colchicine with its sulfhydryl groups (Champanerkar *et al*.,2010). These plants are widely distributed and can be easily propagated through stem cutting.

3.1.6 CHITRAK - *Plumbago zeylanica*

Taxonomic hierarchy

Kingdom:	Plantae
Division:	Tracheophytes
Class :	Eudicots
Order:	Caryophylales
Family:	Plumbaginaceae
Genus:	*Plumbago*
Species:	*zeylanica*

Plumbago is one of the multipurpose medicinal herbs widely distributed in tropics and sub tropics, mostly growing in deciduous woodland and scrublands. Different plant parts contain different chemical compounds like alkaloids, steroids, tannins, triterpenoids, naphthaquinones, glycosides and various oils and fats (Mandavkar YD *et al*.,2011). Among all Plumbagin (5-hydroxy-2-methyl-1, 4-naphthoquinone- C11H8O3) is the principal active compound present richly in roots (Vishnukanta *et al*.,2010) It also shows antibacterial activity against both gram-positive and gram-negative bacteria. Due to various biological activities it is traded worldwide as Ayurvedic and homeopathic medicine. This plant is propagated by seeds, rooted shoots or by semi-ripe cuttings. It prefers well drained loam to clay loam soil along with partially shaded with intermediate warm temperature for better growth. Now a days for mass propagation and production in vitro mass multiplication is for culture of nodal explant, root and leaf explants.

3.1.7 *Typhonium trilobatum*

Taxonomic hierarchy

Kingdom:	Plantae
Division:	Tracheophyta
Class:	Liliopsida
Order:	Alismatales
Family:	Araceae
Genus:	*Typhonium*
Species:	*trilobatum*

These are traditional edible plants of tribes as a good dietary source along with disease curing ability. In India these are grown largely in north eastern states. It is found that it contains phytoelements for body defensive mechanism, nutritional purpose and other anti-nutritional phytoelements. Its roots and tubers contains many important sterols like beta-sitosterol (Banerjee *et al*.,2015). Leaves contains two important anti-nutritional's like phytate and oxalate. The extract of aerial parts with chloroform and ethyl acetate shows anti-inflammatory, anti-bacterial, anti-fungal and anti-diabetic activity (Shaf *et al.*,2005).

3.1.8. GILOY - *Tinospora cordifolia* (Willd.) Miers ex Hook. F.

Taxonomic hierarchy

Kingdom: Plantae
Division Magnoliophyta
Class: Magnoliopsida
Order: Ranunculale
Family: Menispermeacea
Genus: *Tinospora*
Species: *cordifolia*

Tinospora is a climbing shrub in deciduous area having potential medicinal use. It is widely distributed throughout India, China, North west and parts of south east Asia. Its stem contains **Berberine,** 18-norclerodane glucoside,Palmatine, Cordifolisides A to E, Furanoid ditepene glucoside (Nadkarni *et al.*,2010), bark also have various chemical metabolites like **Berberine**, , 18-norclerodane glucoside,Cordioside, Furanoid ditepene glucoside, Palmatine, Palmatosides C and F, Cordifolisides A to E (Sharma *et al.*,2010). The roots are having different combination of drugs having anti-neoplastic and anti-oxidant properties are Jatrorrhizine, Isocolumbin, Tetrahydropalmaitine, Magnoflorine, Palmatine, Tembetarine (Sharma *et al*.,2005). The principal active component of this plant is Berberine an alkaloids along with, it also contains diterpenoid, lactones, glycosides, sesquiterpenoid and other steroids which have anticancer, neurological immunomodulatory, vasorelaxant, psychiatric activities (Rout,2006). This plant can be propagated by stem cuttings or through seeds. However, vegetative method of propagation is used for commercial purpose.

3.1.9 PAN - *Piper betle* Linn.

Taxonomic hierarchy

Kingdom:	Plantae
Division:	Magnoliphyta
Class:	Magnolipsida
Order:	Piperales
Family:	Piperaceae
Genus:	*Piper*
Species:	*betle*

Piper is an annual creeper generally grown in hotter and damper parts of the country. Extensively found is Asia and south east Asia, in India it is found in Uttar Pradesh, Bihar, Bengal, Odisha etc. it is more commonly used in ayurvedic medicines as adjuvant or in combination with other compositions (Bhattacharya *et al.*,2007). It has been described in SUSRTASAMHITA as aromatic, hot, sharp and acrid having valuable use for voice, appetizer and laxative (Verma *et al*.,2010). Its leaves contains Piperol-A, Piperol-B, methyl piper betlol as active ingredient and other metabolites like terpinen-4-ol, allyl pyrocatechol monoacetate, safrole, eugenol, hydroxyl chavicol, eugenyl acetate and eugenol(Chopra *et al.*,1956). This grows very well in tropical and sub-tropical climate with high rainfall and shady places. It can be grown in wide range of soils from sandy loamy to heavy clayey loam.

3.1.10 *Ayapana triplinervis* (M.Vahl) R.King & H.Robinson

Taxonomic hierarchy

Kingdom	Plantae
Division:	Angiosperms
Class:	Asterids
Order:	Asterales
Family:	Asteraceae
Genus:	*Ayapana*
Species	*triplinervis*

Ayapana is a long slender shrub found in tropical areas. It is used by tribes as stimulant, tonic and laxative. Its leaves contains many essential oils like, ayapanin and ayapin, stigmasterol, esculetin methylene ether (the Methylene ether of esculetin) having antiseptic, haemostatic, antibacterial and antifungal activity (Anne GF *et al*.,2008).

3.2 ORNAMENTAL PLANT SELECTED FOR STUDY

***Bougainvillea glabra* L.**

It is an evergreen versatile plant which grows as a vine with stiff thorns and varied colored flowers which makes garden more colorful and attractive. It requires full sunshine and grows better in tropical and subtropical condition. It is used in varied ways like as bush, climber, shrub, in flowering pots or hanging baskets etc. The flowers are bracts and are found in varied colours.

3.3 COLLECTION OF SAMPLES FOR EXTRACT

Medicinal plant samples were collected from Botanical Garden of Regional Plant Resource Centre, Bhubaneswar.

3.3.1 SELECTION OF SOLVENT FOR EXTRACTION

As we had taken ten different medicinal plants having different chemical constituent, each had separate solubility and specificity. So, it is necessary to identify common solvent suitable for all for their extraction. For that we had taken 1 g of each sample is separate test tube containing ethanol, methanol, hexane and acetone as solvent for testing. Form which we derive that methanol is a suitable solvent.

Table. 3. Selection of solvent for extraction (x marked as no desirable; tick marked as desirable)

Name of medicinal plants	Ethanol	Methanol	Hexane	Acetone
Nyctanthes arbortristis linn	√	√	x	x
Moringa oleifora	x	√	√	x
Piper betle	x	√	x	√
Azadirachta indica	√	√	x	x
Mimosa pudica L.	x	√	√	x
Ayapana triplinervis	√	√	x	x
Plumbago zeylanica	x	√	x	√
Tinospora cordifolia	x	√	√	x
Typhonium trilobatum	x	√	x	x
Ocimum sanctun	√	√	√	√

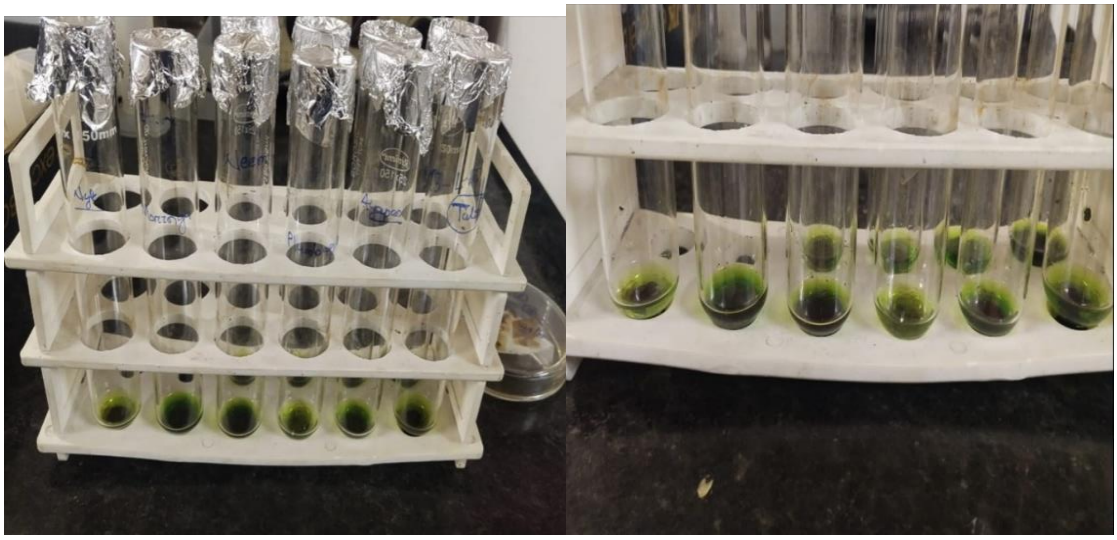


Figure 1. Testing of solvent for extraction of medicinal plant

3.3.2 PREPARATION OF EXTRACT FROM THE LEAF SAMPLES

All the leaves of collected plant samples were first air dried and powered coarsely in mortar and pestle. Then, 5gm of each leaves powder was loaded in the inner tube of the Soxhlet apparatus one by one. After that the apparatus was fitted in to a methanol containing round bottomed flask. Then the solvent was boiled gently (60-80^{0}C) over a heating mantle using the adjustable rheostat. The extraction was continued until complete extraction was complete. The extract was filtered through Whatman filter paper (No.1). After that the extracted solvent was removed and air dried to obtain the viscous dark green or brown residue. That residue was dissolved in methanol and was further used for HPTLC analysis and for treatment of stem cuttings.

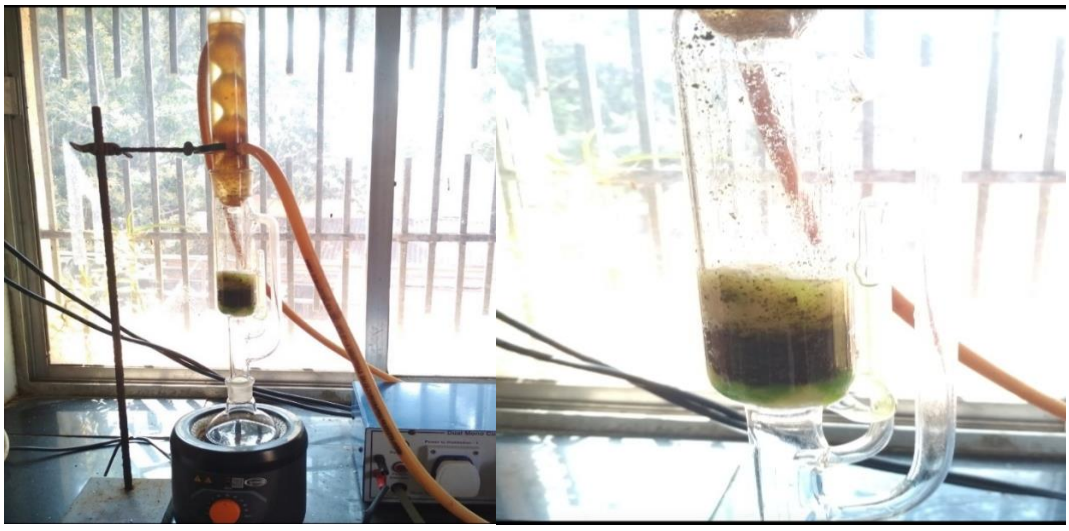


Figure 2. Extraction of medicinal plants through Soxhlet apparatus

3.4 PREPARATION OF STEM CUTTINGS

Healthy and uniform semi hard wood cuttings of pencil thickness was prepared from *Bougainvillea glabra* plant. The diameter and length of these cuttings were kept 2-4 mm and 10-12 cm respectively. To increase the surface area for absorbing the treatment the cuttings were slanted cut at the base. After that sand, soil and vermicompost was mixed in 1:1:1 ratio for preparation of media for planting.

3.5 PREPARATION OF STOCK SOLUTION

For dipping the stem cuttings, the growth regulators stock solution was prepared.

3.5.1 STOCK SOLUTION OF IBA

For preparing stock solution, 400 mg of IBA was taken in a 100 ml flask. First it was dissolved with little amount of ethyl alcohol and then the volume of the solution was raised to 100 ml with addition of distilled water. If necessary, few drops of ammonia can be added to avoid precipitation. From this 4000ppm solution 3000ppm, 2000ppm and 1000ppm solutions were prepared.

3.5.2 STOCK SOLUTION OF IAA

Similarly, stock solution of IAA was prepared by adding 400 mg of IAA with ethyl alcohol for dissolution in a 100 ml flask and after that the volume was raised to 100 ml. Further, it made up to 1000, 2000, 3000 and 4000ppm concentration.

3.5.3 STOCK SOLUTION OF PLANT EXTRACT

The viscous residue was air dried and the powdered form was collected. Stock solution was prepared in similar concentration with growth hormone and was stored for further use. The desired concentration of solution can be prepared from this stock solution by the following formula: Volume 1 x Concentration 1= Volume 2 x Concentration 2. Now the stock solution was stored safely in a closed container in cool condition for further future use.

3.6 TREATMENT OF STEM CUTTING

The remaining leavers are removed from the cutting and dipped in respective solution. Various concentration of IAA and IBA was prepared separately. For dipping of the stem cutting, we had taken 4(25,50,75 and 100%) different concentration

solution for both standard (IAA and IBA) and medicinal plant extract. Each cutting was dipped for period of 24 and 48hrs in each treatment. Three replication was taken for each treatment. After that those cuttings are transferred to pots.

Figure 3. Treatment of stem cuttings in medicinal plant extract

Figure 4. planting of treated stem cutting in potted mixture

3.7 OBSERVATIONS TO BE TAKEN

1) Percentage shoot emergence: We recorded the number of cuttings emerged out in each treatment at weekly intervals.

2) Number of shoots: We noted the emergence of the number of shoots per cutting in each treatment at weekly intervals.

3) Average shoot length: We measured the average shoot length of all the cuttings was by using a measuring scale at weekly intervals.

4) Number of leaves: We observed the number of leaves emerged per cutting at weekly intervals.

5) Number of branches: We noted the number of branches per cutting at weekly intervals.

6) Number of cutting exhibiting rooting: We also counted the number of cuttings exhibiting rooting.

7) Per cent of cutting exhibiting rooting: We calculated the total per cent of cutting exhibiting rooting performance.

8) Number of roots emerged: For recording the number of roots emerged, the cuttings were uprooted very carefully without damaging to root system. After that the roots of each cutting were thoroughly washed in running tap water to remove sand for taking observations.

9) Average length of roots: The roots were removed from cutting with help of a sharp razor. The length of primary roots of each cutting was recorded.

10) Per cent establishment of plants: Per cent survival of cuttings was recorded in each treatment after four months by counting the total number of surviving plants out of number of treated cuttings.

3.8 HPTLC ANALYSIS

We purchased the prepared TLC plates from EMerck (Damstadt, Germany). The required standards growth hormones were obtained.

3.8.1 SELECTION OF SOLVENT SYSTEM AS A SUITABLE MOBILE PHASE

The mobile phase for HPTLC analysis differs from plant to plant based on their chemical constituent. So, as we had ten different plants having different active chemical constituents we selected a suitable mobile phase, for all through TLC analysis checking the band formation. For that we had taken ten different solvent system such as propanol: water (8:2), butanol: glacial acetic acid: water (12:5:3), ethyl acetate: methanol: water (40:6: 4), toluene: ethyl acetate: water (20: 15: 5) and toluene: ethyl acetate: methanol: formic acid (20: 15: 10: 5). The suitable solvent system was decided as ethyl acetate: methanol: water (40:6: 4).

Table 4. Selection of mobile phase for HPTLC analysis

Name of the plant	Active compound	TLC analysis				
		Solvent-1	Solvent-2	Solvent-3	Solvent-4	Solvent-5
		Propanol: water (8:2)	butanol: acetic acid: water (12:5:3)	Ethyl acetate: methanol: water (40:6:4)	toluene: ethyl acetate: water (20:15:5)	toluene: ethyl acetate: methanol: formic acid (20:15:10:5)
IAA				√		√
IBA				√		
Nyctanthes	nicotiflorin, nyctanthic			√	√	
Moringa oleifera	niazimicin			√		√
Piper betle	Piperol-A, Piperol-B,					
Azadirachita indics	Nimbidin, Nimbin		√	√		
Mimosa pudica	mimosine, mimosinamine, mimosinic acid	√		√		
Ayapana triplinervis	ayapanin and ayapin			√	√	
Plumbago	Plumbagin		√	√	√	
Tinospora	Berberine			√		√
Typhonium	beta-sitosterol	√		√		
Ocimum	Eugenol			√	√	

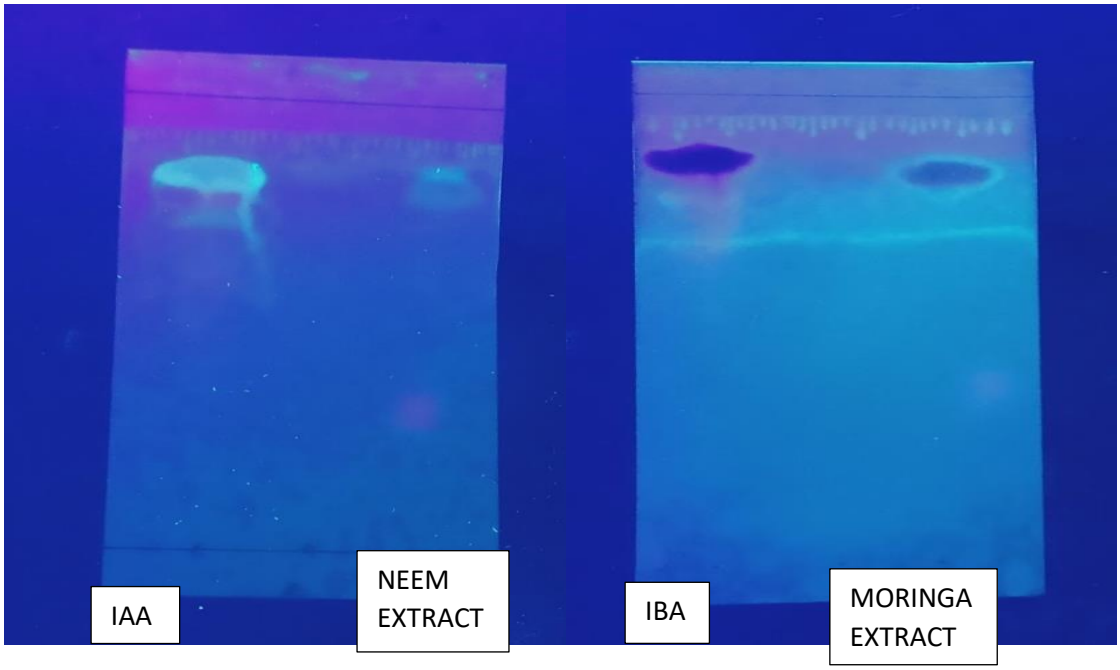


Figure 5. TLC of growth hormone and plant extract for selection of mobile phase

3.8.2 DEVELOPING SOLVENT SYSTEM

A number of solvent systems were tried for deriving a suitable composition suitable for all plant sample and growth hormone. But the satisfactory resolution was obtained in the solvent Ethyl acetate: methanol: water (40:6:4).

Figure .6. Development of solvent system

3.8.3 SAMPLE APPLICATION FOR CHROMATOGRAPHIC ANALYSIS

We used aluminium plate precoated with silicagel 60F254 TLC plates (10 x 10) as the stationary phase. The samples and standards were loaded on the TLC plate by a Linomat IV (Camag, Muttenz, Switzerland) automatic TLC applicator which works under a flow of nitrogen gas. The delivery speed of the syringe was 10s/micro ml. all the analysis had similar application parameters with identical condition. The standards and samples solutions were prepared in HPLC grade methanol. The extracts and standards were applied on the plate about 1-1.5 cm away from the lower edge.

3.8.4 DEVELOPMENT OF CHROMATOGRAM

After the application of sample, the chromatogram was prepared in Twin trough glass chamber 10x10 cm. For identification the solvent solution prepared with Ethylacetate: Methanol: Water (v/v/v) (40:6:4) under laboratory condition (25-30^{0}C) and 40-50% relative humidity. The chamber was saturated with solvent for 15 mins. After loading the plates were then placed in that saturated chamber. The plate was taken out from the chamber only after 90% movement of solvent front in the plate.

3.8.5 RESULT DETECTION

After that the plate was dried in room temperature. The plates were scanned by densitometer at 254nm and 366nm. Then these developed plates were derivatized by spraying vaniline solution (100mg vaniline + 28ml methanol + 1 ml H_2SO_4) and again scanned. The Rf value and all finger print data were recorded by WIN CATS software.

3.9 BIOCHEMICAL ANALYSIS

For each method and analysis, five distinct plants from each treatment were detached during the harvesting stage of crop growth. For each observation, there are several replications. The samples were quickly placed in an ice bucket for cooling. The temperature was kept at 4°C throughout the estimation for the enzymatic investigation.

3.9.1 CHLOROPHYLL ESTIMATION:

The method of estimating leaf chlorophyll by using 80% Acetone and light absorption at 663 nm and 645 nm was measured in a spectrophotometer. The amount of chlorophyll in the leaf was calculated by absorption coefficients.

Reagent:

80% Acetone (prechilled prior to experiment)

Method:

(i) A fresh leaf sample of 1gram was collected and cut into pieces.

(ii) Grind the tissue to fine powder by using mortar and pestle with addition of 20ml of 80% Acetone solution.

(iii) Then centrifuge it in 5000 rpm for 5 minutes and transfer the supernatant to a 100ml volumetric flask.

(iv) Again, grind the residue with 20% acetone solution, centrifuge and transfer to same volumetric flask.

(v) Repeat the same procedure until the residue is colourless.

(vi) Collect the clear residue and makeup the volume to 100 ml with 80% Acetone.

(vii) Finally, read the absorption of the solution at 663nm and 645nm against the solvent (acetone) as blank.

The chlorophyll content was calculated using the formula below and given in milli grammes per gram leaf weight (fresh).

mg chlorophyll -a/g of tissue = 12.7(A663)-2.69(A645) ×V/100 ×W

mg chlorophyll -b/g of tissue = 20.2(A645)-4.68(A663) ×V/100 ×W

mg total chlorophyll /g of tissue = 20.2(A645)-8.02(A663) ×V/100 ×W

Where, A is the absorption at specific wavelength.

V is the final volume of chlorophyll extract.

W is the fresh weight of leaf tissue.

3.9.2 ESTIMATION OF PROTEIN BY LOWRY METHOD:

Protein estimation can be done by various methods, Lowry method is the most prominently used method which gives a constant value, hence largely followed.

Reagent:

Reagent-A: 2% sodium carbonate + 0.1 N sodium hydroxide.

Reagent-B: 0.5% copper sulphate + 1% potassium sodium tatarate.

Reagent-C: Alkaline copper solution; mix 50ml reagent A and 1ml reagent B.

Reagent-D: Folin-ciocalteau reagent.

Protein solution (standard)

Method:

(i) Take 5 test tube and pipette out 0.2, 0.4, 0.6, 0.8, and 1ml of working standard.

(ii) Pipette out 0.1 ml and 0.2 ml of the sample extract into the the other test.

(iii) Make up the final volume to 1ml in all the test tube and a blank contains 1ml of water.

(iv) Add 5ml of reagent C in each test tube and allow to stand for 10 minutes.

(v) Then add 0.5ml of reagent D mix well and incubate in dark room for 30 minutes.

(vi) Take the reading at 660nm.Draw the standard graph and calculate the amount of protein content.

RESULT

The result pertaining to the present study entitled "Evaluation of bioactive compounds from selected medicinal plant extract as root promoter potential". The experiment was conducted in the Department of Agricultural Biotechnology, College of Agriculture, Bhubaneswar and presented below. *Bougainvillea glabra* was selected as ornamental plant for testing the effect of treatment of plant extract in comparison to the application of growth hormone for propagation through stem cuttings.

4.1 SHOOT EMERGENCE

The semihard wood cuttings of *Bougainvillea glabra* were treated with medical plants extract and growth hormones for 24 and 48 h duration and observation were recorded after shoot emergence.

Table .5. Effect of different concentrations of plant extract under 24h treatment on shoot sprouting from stem cuttings of *Bougainvillea glabra*.

	Shoot sprouting (%) (24 hrs), (3 replications/treatment)				
Treatments	4000ppm	3000ppm	2000ppm	1000ppm	Days to shoot sprouting
IBA	87.3	80.2	78.4	-	14.2
IAA	80.2	82.5	70.8	-	13
Moringa oleifera	79.4	70.8	65.9	-	17.4
Nyctanthes	-	52.4	50.5	47.8	24.5
Piper betle	80.3	82.3	-	78.4	15.6
Mimosa	-	53.8	-	50.5	22.4
Azadirachta indica	-	-	50.5	49.8	25.7
Plumbago	48.7	-	47.9	-	28.3
Tinospora	62.5	-	-	54.2	25
Typhonium	-	35.5	-	-	32
Ocimum	-	35.7	-	34.8	20.2
Ayapana	-	63.5	-	22.3	22
Control	35.9	-	34.3	-	32

It was observed that stem cuttings treated with growth regulators shows maximum emergence of rooting at 4000ppm and 3000 ppm treatment. Comparing this result with the result of sprouting percentage of stem cuttings treated with different medicinal plant extract, it showed that the leaf extracts of *Moringa oleifera, Piper betle* and *Tinospora* showed significant effect as compared with growth regulators. However, while comparing the duration or time taken for emergence of shoots from the stem cutting only obtained in *Piper betle* and *Moringa oleifera* extracts within 15-17 days. But the stem cuttings treated with *Tinospora* extract showed bud sprouting after 25 days and very close to the control. Hence, it can be stated that *Piper betle* and *Moringa oleifera* leaf extracts have significant influence on bud sprouting as well as root initiation from stem cuttings **(Figure 8).**

Figure 7. Sprouting from treated stem cutting of *Bougainvillea glabra*.

Figure. 8. Bud sprouting from stem cuttings of *Bougainvillea glabra*

Table 6. Effect of different concentrations of plant extract under 48h treatment on shoot sprouting from stem cuttings of *Bougainvillea glabra*.

	Shoot sprouting (%) (48 hrs) (3 replications/treatment)				
Treatment	4000ppm	3000ppm	2000ppm	1000ppm	Days to shoot sprouting
IBA	83.1	78.2	80.4	-	15
IAA	70.5	72.5	70.8	-	16.4
Moringa oleifera	70.4	70.8	-	58.5	18
Nyctanthes	-	-	35.5	37.8	33
Piper betle	65	75.3	72.5	-	17.2
Mimosa	-	-	34.7	32.7	25.7
Azadirachta indica	-	-	38.5	35.7	28.3
Plumbago	42.6	45.6	-	-	30.2
Tinospora	-	58.3	-	54.2	23
Typhonium	-	-	-	38.7	30
Ocimum	-	-	32.5	30.2	22
Ayapana	-	-	30.5	31.3	25
Control	-	-	30.3	30.7	34

The treatment was also continued for 48h duration to check enhancement and retardance of sprouting. It is observed that when the duration of treatment increased there is retardance in percentage of sprouting with increased the concentration. The stem cuttings treated with growth regulators under 48h showed low rate of shoot sprouting which may be causing toxic effect. Similarly, the cuttings treated with plant extract also had significant toxic effect when treated for longer duration. From the above observation it can be noted that *Moringa oleifera* and *Piper betle* leaf extracts may contain some auxin like substances which showed similar effect as that of growth regulators. The results are further confirmed through HPTLC and FTIR analysis.

Table.7. Relationship between concentration of growth hormone application and percentage sprouting

Different concentration of Growth regulators	Sprouting (%)			
	IAA		IBA	
	24 hr	48hr	24 hr	48 hr
4000 ppm	80.2	72.2	87.3	83.1
3000 ppm	82.5	70.5	80.2	78.2
2000 ppm	70.8	70.8	78.4	80.4
1000 ppm	-	-	-	-

The table-7 represent the effect of growth regulators on shoot sprouting from semi hard wood cutting of *Bougainvillea glabra*. It is clearly observed that with increasing the duration of treatment, resulted in significant reduction of sprouting and causing toxic effect. So also, with the treatment of medicinal plant extract containing specific active chemical ingredients.

4.2 ROOT EMERGENCE

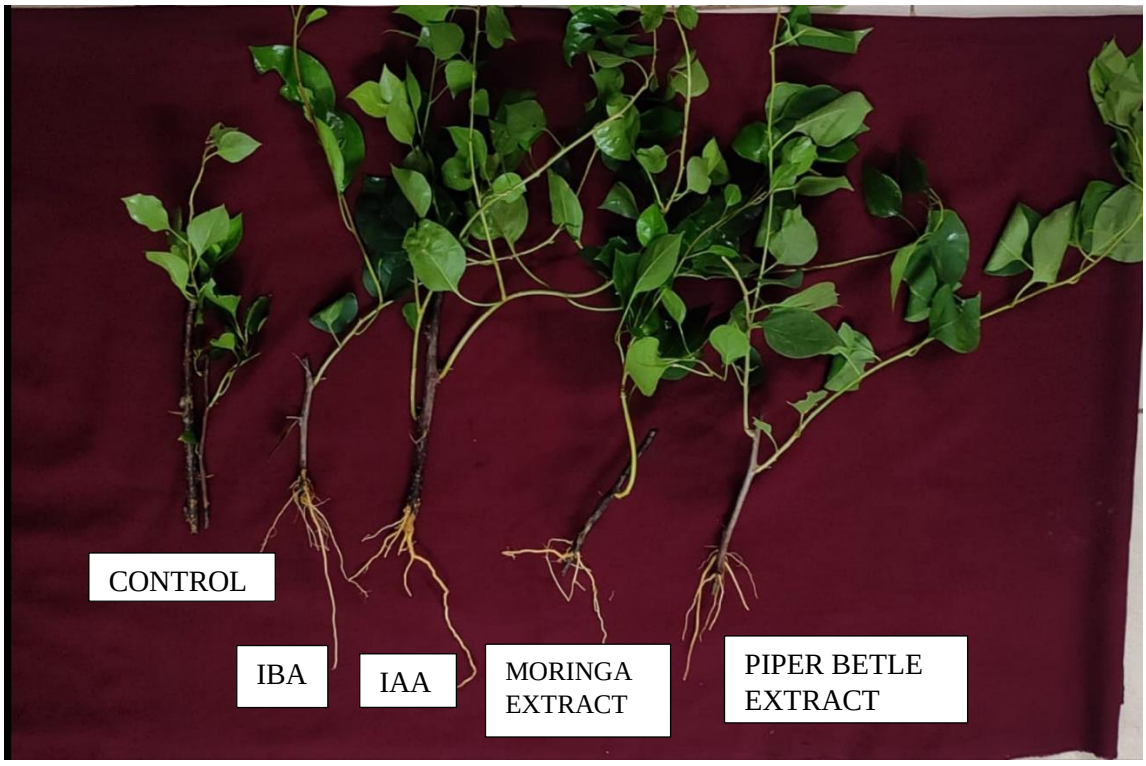


Figure 9. Root emergence from stem cuttings of *Bougainvillea glabra*.

Table 8. Root emergence from stem cuttings of *Bougainvillea glabra* treated with different concentrations of growth regulators and plant extracts. (3 replicates per treatment).

Source of Plant extracts/growth regulators	Concentration	Av. number of roots/stems cutting	
		24 hrs	48 hrs
Control	-	0	0
Moringa oleifera	3000 ppm	1.2	1.4
	4000 ppm	3.4	2.6
Piper betle	3000 ppm	3.2	3.7
	4000 ppm	2.8	1.6
IAA	3000 ppm	3.6	3.8
	4000 ppm	4.2	2.6
IBA	3000 ppm	3.5	3.7
	4000 ppm	4.2	3.4

Table 8 showed the comparison of root emergence in different application of growth regulators and plant extract treatment of *Moringa oleifera* and *Piper betle.* While treatment with *Moringa oleifera* leaf extract showed significant root growth development in 4000 ppm. But, in case of *Piper betle* the root development was observed in 3000 ppm.

Table. 9. Shoot and Root emergence from stem cuttings of *Bougainvillea glabra* treated with different concentrations of growth regulators and plant extracts. (3 replicates per treatment).

Source of Plant extracts/growth regulator	Concentration	Shoot sprouting (%)	Rooting percentage	Av. No. of main roots/cutting	Av. No. of lateral roots/cutting
Moringa oleifera	4000 ppm	74.9	77.1	3.4	2.6
Piper betle	3000 ppm	78.8	75.9	3,2	1.7
IAA	3000 ppm	76.5	78.4	3.5	2.8
IBA	4000 ppm	85.2	82.3	4.1	2.6

While comparing overall growth through percentage of sprouting and percentage of rooting and root development, it was observed that both *Piper betle* and *Moringa oleifera* leaf extract had significant growth effect similar to growth regulators.

4.3 HPTLC ANALYSIS

Medicinal plant extracts and growth hormone standards (auxin: IAA and IBA) were run on the HPTLC plates, for comparing the presence of auxin or auxin like substances in plant extracts. After the plate was dried in room temperature and scanned

by densitometer at 254nm and 366nm. Then these plates were derivatized by spraying vaniline solution (100mg vaniline + 28ml methanol + 1 ml H_2SO_4) and again scanned. The R_f value and the finger print data were analyzed by using WIN CATS software.

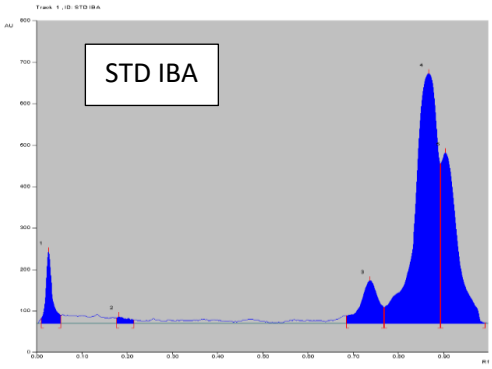
Development of stain in the chromatographic plate represents the movement of solvent in the stationary phase.

Figure .10. Chromatographic plate after derivation

The chromatographic plate was scanned in two different wave length i.e. 254 nm and 366 nm. The plate was scanned before and after derivation so that we can derive appropriate result by comparing and cross checking the data obtained at different wavelength.

4.3.1 COMPARISON

After derivation, the individual graph of medicinal plant extract was compared with the graph of growth hormones. The Rf value, its corresponding peak, range, area coverage of the peak and maximum percentage of content present in that peak was taken as parameter for comparison.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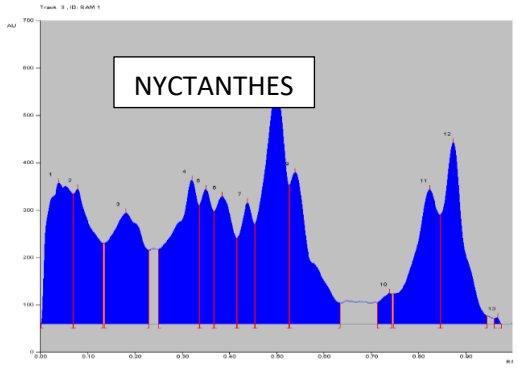
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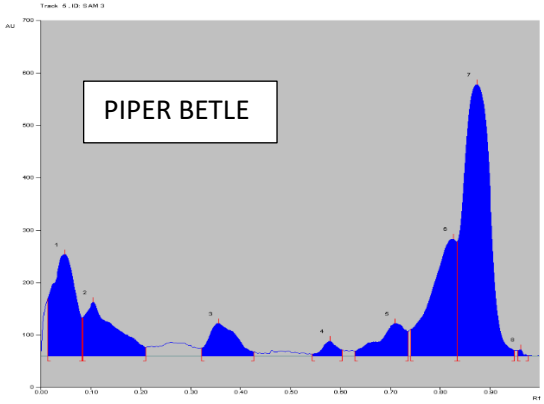
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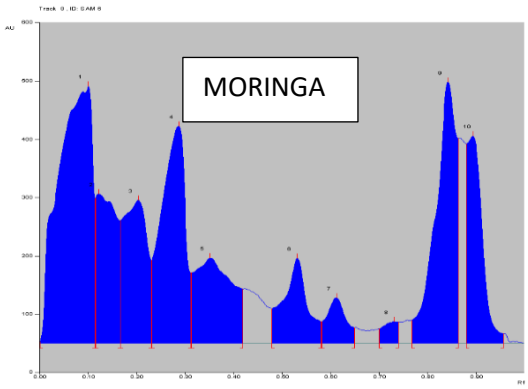
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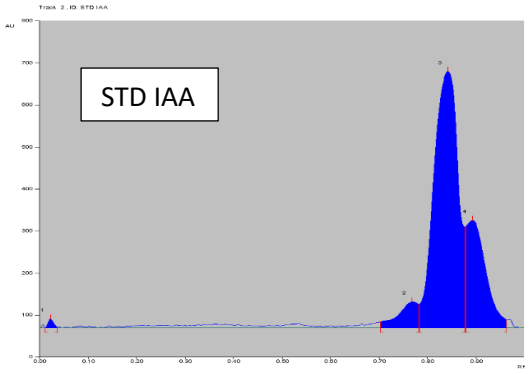
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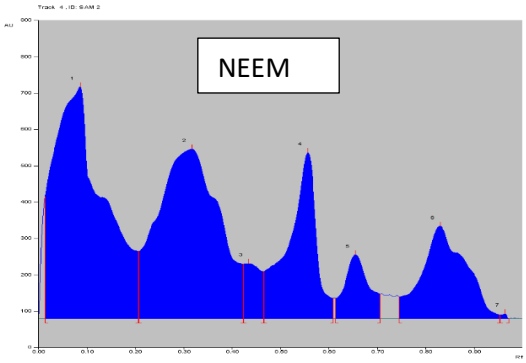
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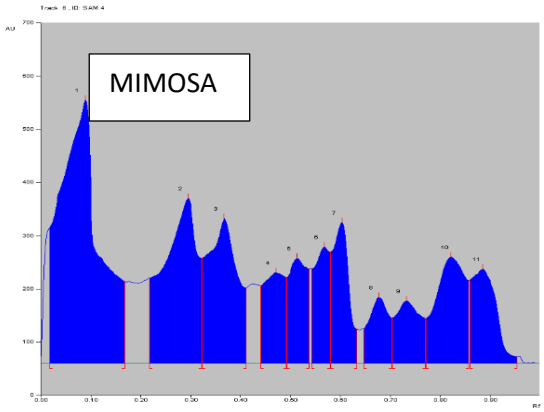
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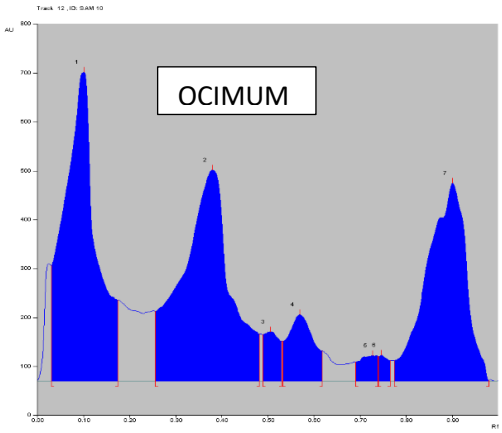
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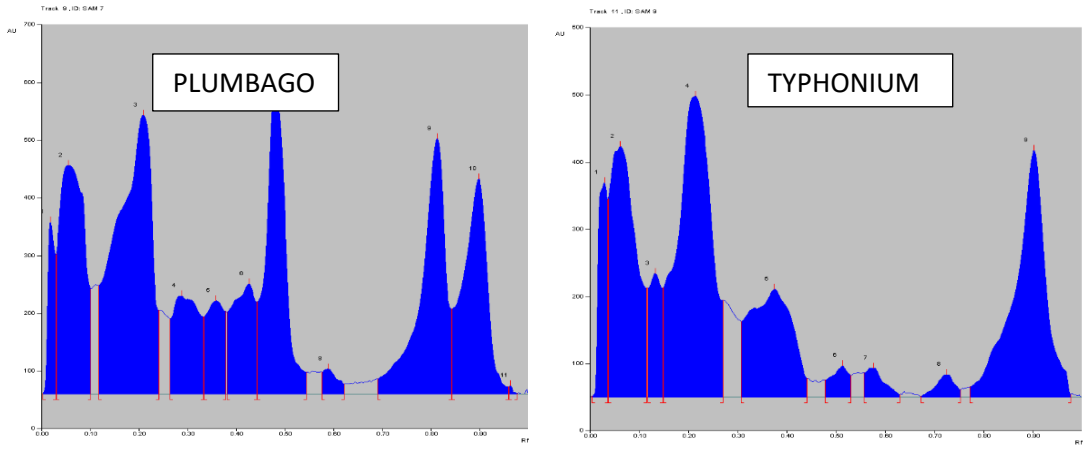

Figure 11. comparison of individual pic of Rf of each medicinal plant extract

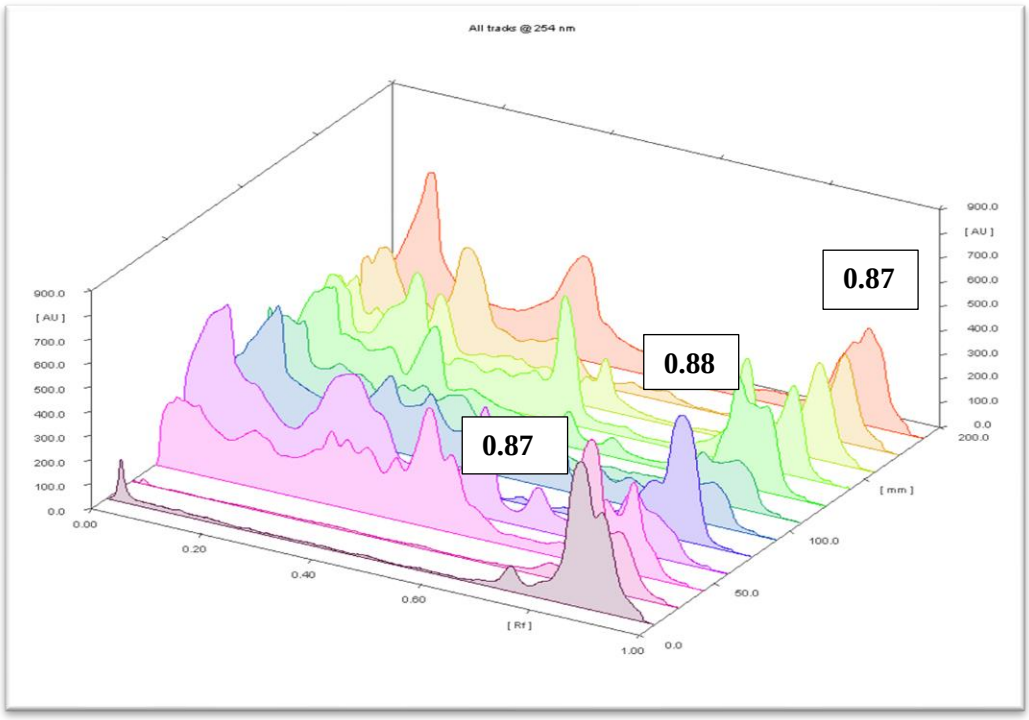

Figure 12. 3D graph HPTLC at 254nm wavelength before derivation, solvent present are IBA, IAA, *Nyctanthes, Moringa oleifera, Piper betle, Azadirachta indica, Mimosa, Ayapana, Plumbago, Tinospora, Typhonium and Ocimum* respectively.

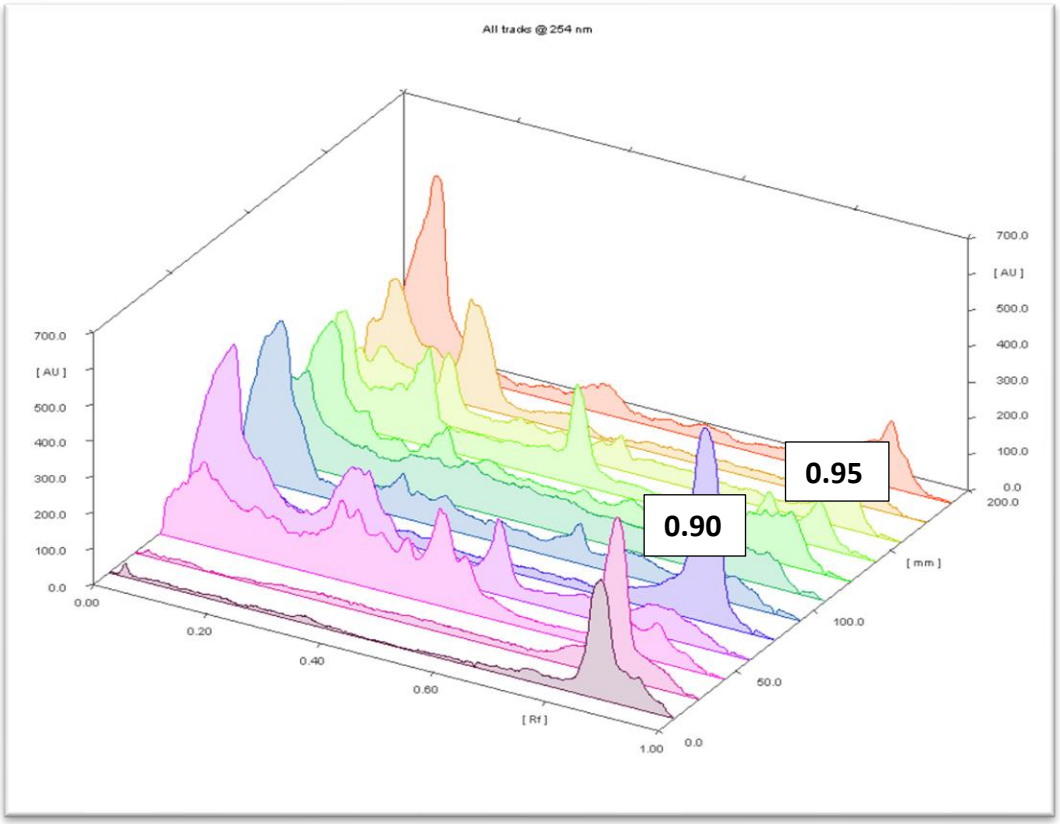

Figure 13. 3D graph HPTLC after derivation at 254 nm wavelength, solvent present are IBA, IAA, *Nyctanthes*, *Moringa oleifera*, *Piper betle*, *Azadirachta indica*, *Mimosa*, *Ayapana*, *Plumbago*, *Tinospora*, *Typhonium* and *Ocimum* respectively.

Table 10. Comparative study of data obtained at 254 nm

	Observation at 254nm without derivation			Observation at 254nm after derivation		
Samples (4000ppm)	Range (Rf)	Max Conc. (%)	Area (cm^2)	Range (Rf)	Max Conc.(%)	Area (cm^2)
STD IBA	0.77-0.89	46.12	27385.1	0.81-0.91	61.78	13609.1
STD IAA	0.78-0.88	64.29	28443.2	0.76-0.92	86.43	22016.7
Nyctanthes	0.85-0.94	10.98	14452.4	0.78-0.85	4.72	4181.1
Moringa oleifera	0.75-0.95	31.81	21152.7	0.79-0.85	4.63	4156.5
Piper betle	0.83-0.95	43.05	24835.7	0.77-0.95	64.71	28726.4
Azadirachta indica	0.77-0.86	7.84	10810.8	0.78-0.86	7.01	4943.4
Mimosa	0.84-0.95	6.25	11399.6	0.88-0.95	5.91	3367.7
Ayapan	0.77-0.86	17.73	17781.2	0.88-0.95	9.87	5117.4
Plumbago	0.84-0.96	12.01	16870.6	0.84-0.94	8.08	6046.4
Tinospora	0.82-0.98	16.39	22234.8	0.84-0.96	11.93	8364.1
Typhonium	0.77-0.98	18.58	21413.5	0.83-0.94	11.48	5961.6
Ocimum	0.78-0.98	22.38	32029.3	0.81-0.96	15.45	11185.5

The above table represents data of HPTLC finger print scanned at 254 nm wavelength before and after derivation. Comparing the Rf value range, percentage of

maximum concentration of substrate at that range and the area coverage of peak we can derive which plant extract contains auxin concentration similar to that of standard.

Analyzing the above table, it was observed that the leaf extracts of *Moringa oleifera*, *Piper betle*, *Tinospora*, and *Ocimum* showed similar peak in Rf range with standard growth regulators. However, only *Piper betle* shows maximum concentration of substrate in that Rf range followed by *Moringa oleifera*, *Ocimum*, *Typhonium* and *Tinospora*.

After derivation, when again analyzed in wavelength of 254 nm, it was observed that only *Piper betle* extract showing similar peak with higher area coverage and higher percentage of substrate concentration.

Thus, from above analysis it was noted that *Piper betle* and *Moringa oleifera* may have auxin or auxin like substances which can be used as growth hormone substitute.

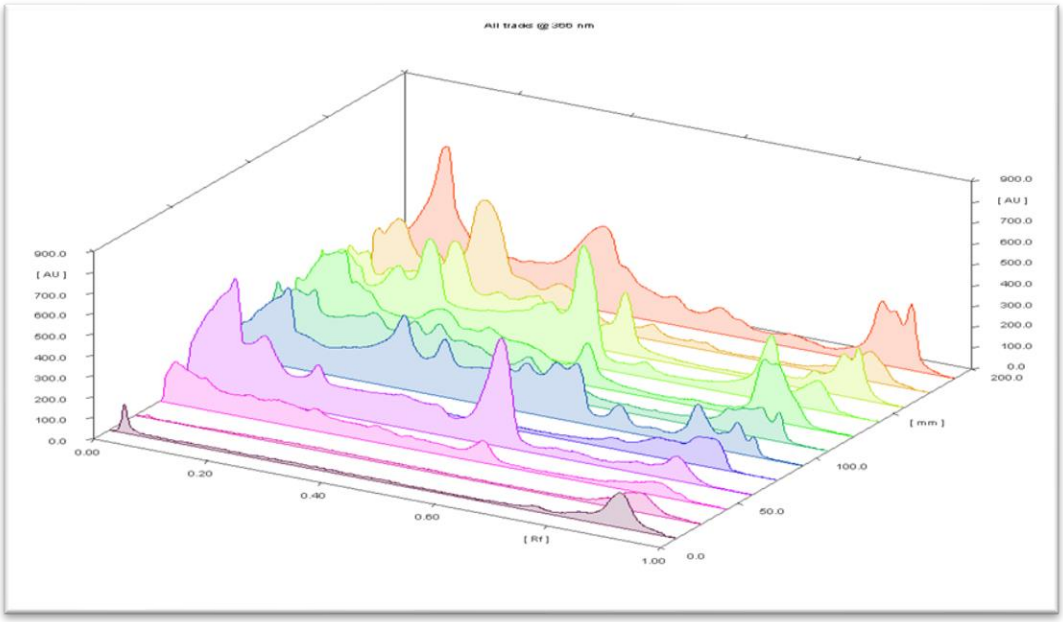

Figure 14.3D graph HPTLC at 366nm before derivation, solvent present are IBA, IAA, *Nyctanthes, Moringa oleifera, Piper betle, Azadirachta indica, Mimosa, Ayapana, Plumbago, Tinospora, Typhonium* and *Ocimum* respectively.

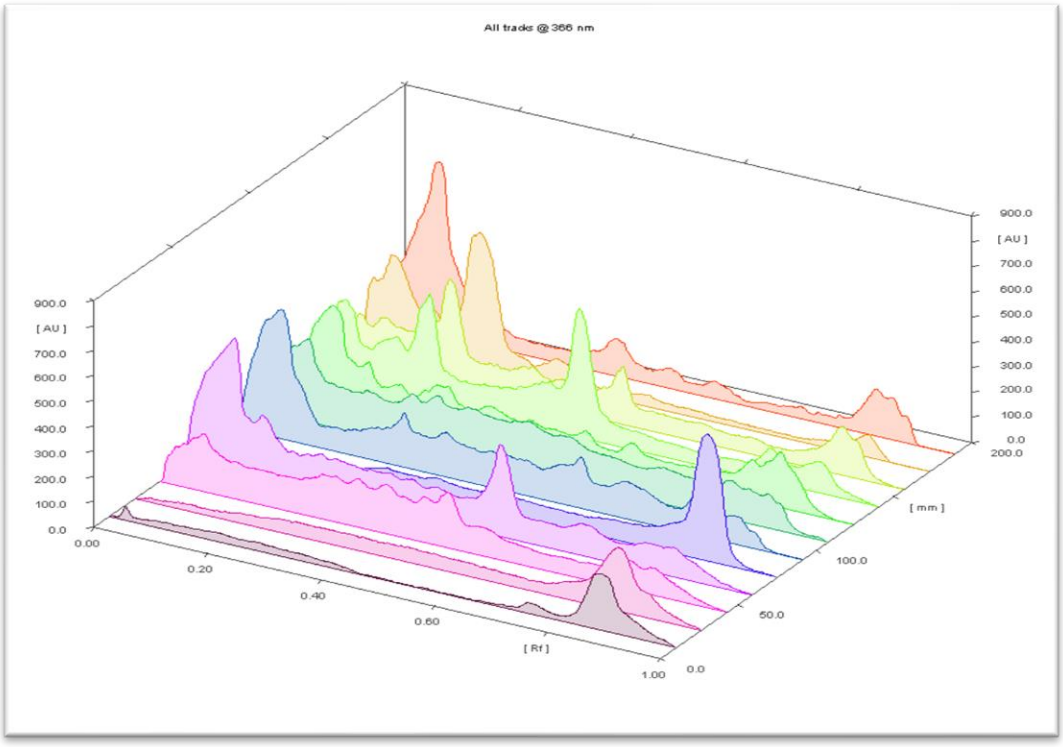

Figure 15.3D graph at 366 nm wavelength after derivation, solvent present are IBA, IAA, *Nyctanthes, Moringa oleifera, Piper betle, Azadirachta indica, Mimosa, Ayapana, Plumbago, Tinospora, Typhonium* and *Ocimum* respectively.

Table 11. Comparative study of data obtained at 366nm

	Observation at 366 nm without derivation			Observation at 366 nm after derivation		
Samples(4000ppm)	Range (Rf)	Max (%)	Area (cm^2)	Range (Rf)	Max (%)	Area(cm^2)
STD IBA	0.83-0.97	50.59	8438.0	0.80-0.97	60.47	13886.2
STD IAA	0.80-0.96	87.59	6157.4	0.78-0.94	64.34	18113.7
Nyctanthes	0.82-0.94	7.04	4328.0	0.79-0.85	5.83	5147.0
Moringa oleifera	0.82-0.93	5.28	5289.0	0.85-0.95	4.45	5883.9
Piper betle	0.83-0.92	21.42	7872.2	0.79-0.95	63.82	26973.1
Azadirachta indica	0.85-0.90	5.74	7422.7	0.79-0.87	8.71	9309.7
Mimosa	0.86-0.90	5.35	4747.3	0.79-0.84	6.00	5887.2
Ayapan	0.86-0.93	12.03	8879.7	0.84-0.97	10.79	11558.3
Plumbago	0.85-0.95	5.05	6349.7	0.86-0.96	5.25	6481.2
Tinospora	0.86-0.91	7.29	6083.6	0.80-0.95	7.94	12453.8
Typhonium	0.81-0.96	7.88	8933.1	0.84-0.92	4.96	3847.3
Ocimum	0.82-0.89	11.43	9621.5	0.81-0.88	12.31	6920.2

Table 11contains information recorded over 366 nm with and without derivation. Analysis of the above data shows similar area coverage within the similar Rf range with standard. So taking percentage of maximum concentration of substrate for comparison we found only *Piper betle* having significant presence.

When again analyzed after derivation we found *Piper betle, Ayapana* and *Tinospora* having maximum peak area in the Rf range but when we observe the percentage of maximum concentration, it was observed that only *Piper betle* having significant result.

From both analysis this is confirmed that *Piper betle* leaves and *Moringa oleifera* leaves have significant amount of auxin like substances or auxin followed by *Ocimum* and *Ayapan*a which can be used as substitute of synthetic growth hormone.

4.4 CHLOROPHYLL ESTIMATION

Table. 12 Chlorophyll content in leaves of sprouted stem cuttings

Treatments	Chlorophyll a	Chlorophyll b
Control	0.0143	0.0045
IAA	0.0200	0.0070
IBA	0.0315	0.0104
Moringa extract	0.0218	0.0070
Piper betle extract	0.0277	0.0092
Tinospora extract	0.0192	0.0068

Estimating the chlorophyll content we found that *Moringa oleifera* and *Piper betle* have similar activity as that of plant growth hormones and hence confirming similar biochemical activity as that of growth hormone.

4.5 FTIR ANALYSIS

For further more specific detection FTIR analysis was done for deriving specific function group present in the plant extract.

4.5.1 FTIR

FTIR stands for Fourier Transform Infrared, is one of the most preferred method of infrared spectroscopy. This is used for identification of organic compounds, polymers and other inorganic constituents. It works on the principle of NDIR. Each organic or inorganic compound absorbs infrared radiation at specific frequencies, which can be measured to identify specific compound. Thus, it is one of the rapid, time saving, nondestructive and efficient method which is widely used to detect the presence of range of functional groups and is sensitive to change in molecular structure.

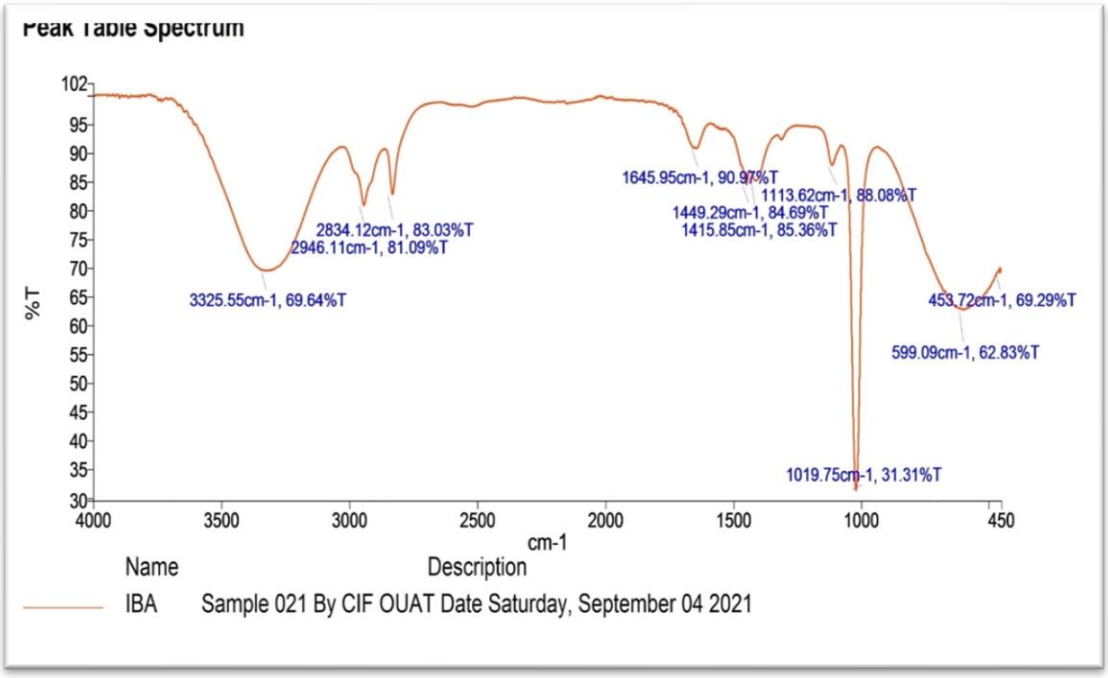

Figure 17. FTIR graph of IBA

Table. 13. Functional group present in IBA

Frequency (cm^{-1})	Reference frequency range (cm^{-1})	Functional group
3325.55	3200-3550	OH streach, Alcohol group
2946.11	2800-3000	N-H bond, amine group
2834.12	2800-3000	N-H bond, amine group
1645.95	1640-1690	C=N, imine/oxime
	1626-1662	C=C, alkene
1449.29	1450	C-H bond, alkane
1415.85	1330-1420	O-H bond, alcohol
1113.62	1087-1124	C-O bond, s-alcohol
1019.75	1020-1250	C-N bond, amine
599.09	515-690	C-Br bond,
	500-600	C-I bond

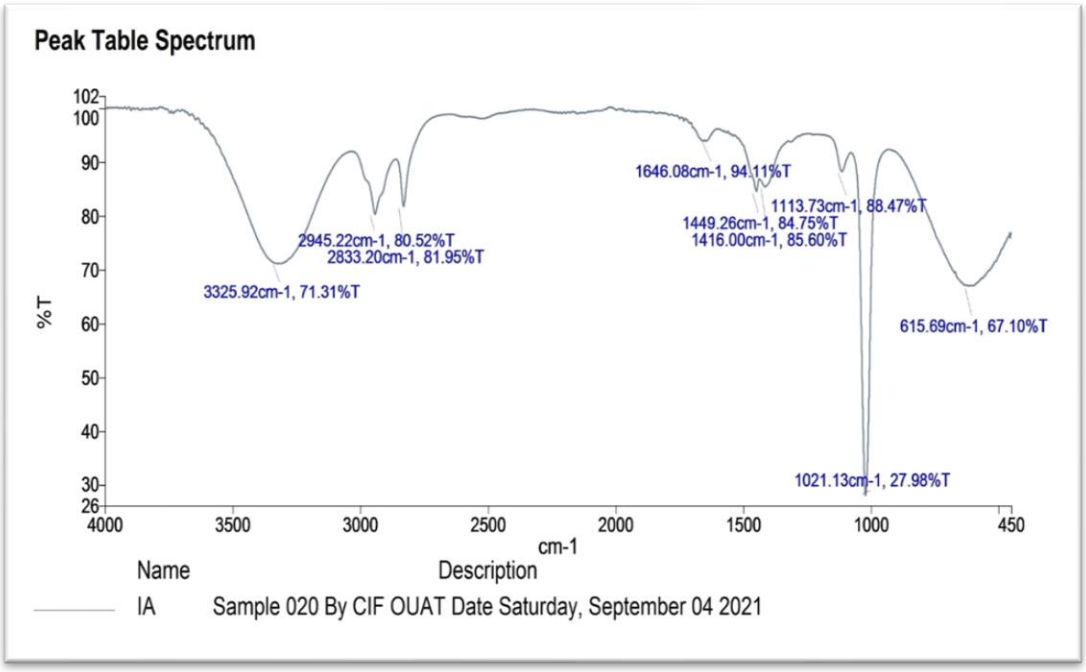

Figure 18. FTIR graph of IAA

Table. 14. Functional group present in IAA

Frequency (cm^{-1})	Reference frequency range (cm^{-1})	Functional group
3325.92	3200-3550	OH streach, Alcohol group
2945.22	2800-3000	N-H bond, amine group
2833.20	2800-3000	N-H bond, amine group
1646.08	1640-1690	C=N, imine/oxime
	1626-1662	C=C, alkene
1449.26	1450	C-H bond, alkane
1416	1330-1420	O-H bond, alcohol
1113.73	1087-1124	C-O bond, s-alcohol
1021.13	1020-1250	C-N bond, amine
615.69	515-690	C-Br bond,
	500-600	C-I bond

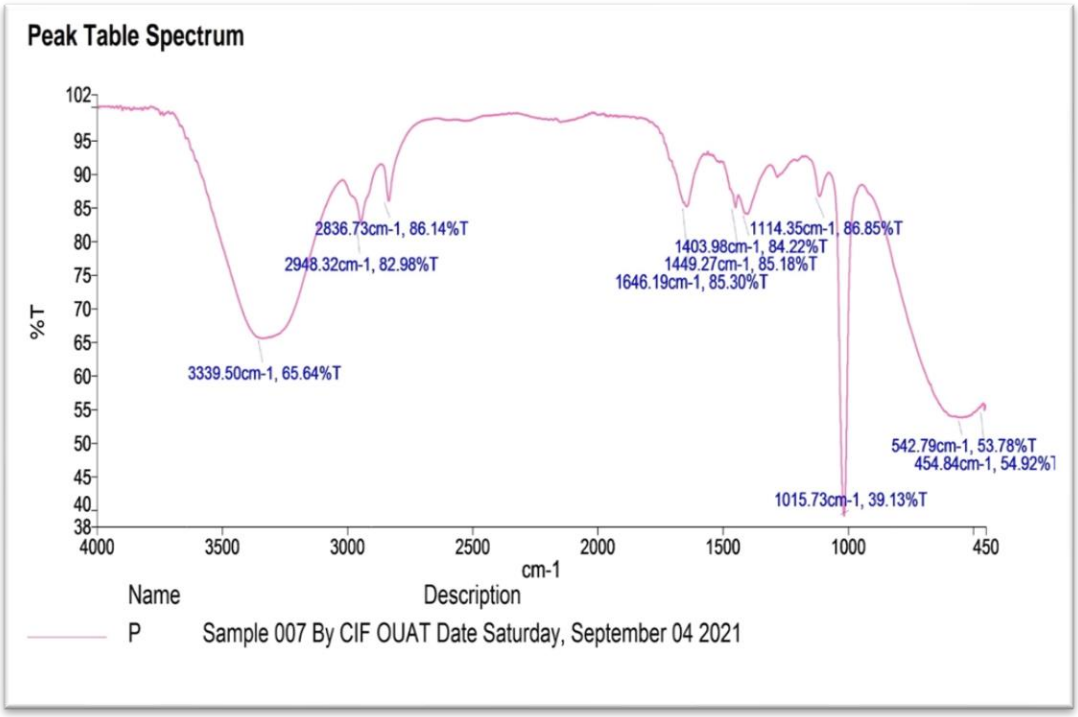

Figure 19. FTIR graph of *Piper betl*e leaf extract

Table. 15. Functional group present in *Piper betle* leaf extract

Frequency (cm^{-1})	Reference frequency range (cm^{-1})	Functional group
3339.50	3200-3550	OH streach, Alcohol group
2948.32	2800-3000	N-H bond, amine group
2836.73	2800-3000	N-H bond, amine group
1646.19	1640-1690	C=N, imine/oxime
	1626-1662	C=C, alkene
1449.27	1450	C-H bond, alkane
1403.98	1395-1440	O-H bond, Carboxylic acid
1114.35	1087-1124	C-O bond, s-alcohol
1015.73	1020-1250	C-N bond, amine
542.79	515-690	C-Br bond,
	500-600	C-I bond

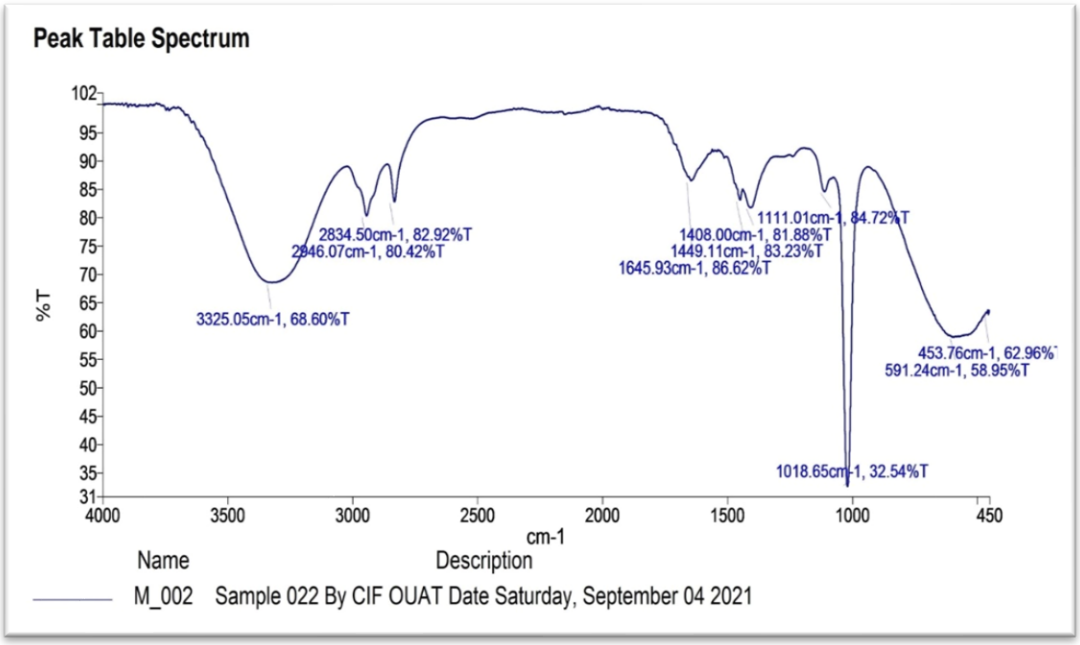

Figure 20. FTIR graph of *Moringa oleifera* leaf extract

Table. 16. Functional group present in *Moringa oleifera* leaf extract

Frequency (cm^{-1})	Reference frequency range (cm^{-1})	Functional group
3325.05	3200-3550	OH streach, Alcohol group
2946.07	2800-3000	N-H bond, amine group
2834.50	2800-3000	N-H bond, amine group
1645.93	1640-1690	C=N, imine/oxime
	1626-1662	C=C, alkene
1449.11	1450	C-H bond, alkane
1408.00	1395-1440	O-H bond, Carboxylic acid
1111.01	1087-1124	C-O bond, s-alcohol
1018.65	1020-1250	C-N bond, amine
591.24	515-690	C-Br bond,
	500-600	C-I bond

The following FTIR analysis shows very similar chemical constituent with that of growth hormone. Plant extract of *Moringa oleifera* and *Piper betle* contains similar organic compound containing amine, imine, alcohol and carboxylic group. From the above analysis it can be derived that leaf extract of *Moringa oleifera* and *Piper betle* contains auxin or auxin like substances and thus can be used as growth hormone substitute.

DISCUSSION

Medicinal plants are rich source of different effective chemical compounds which are used by human beings from many decades. These are directly taken as food and also in concentrated from as medication. India with its rich diversity is a hotspot of many medicinal plant species. The subtropical and temperate climate provides a perfect growing condition for the development of these medicinal plants. Adequate sunlight, moisture and temperature increases the active chemical constituents of the plants. Thus, these are used as raw material in pharmaceutical industries manufacturing of medicines. However, these active constituents also have different anti healing, antibacterial, antimicrobial and many more efficient activities, which can be further analyzed and utilized for other purposes.

Bougainvillea glabra is one of the ornamental plant most widely used for decoration and avenue purpose. These are mostly used in fencings, in hedge making, and also in between high ways due to its hardy nature and capacity to absorb the pollutants. These are grown in pots and also in hanging baskets for decoration purpose. These are environmental tolerance and can grow in drought condition also. Thus, these are commercially used and propagated by semihard wood cuttings of plants. In general, for early and effective establishment of stem cuttings generally growth hormones are applied. However uncontrolled use and excess application may cause toxic effect. So also, there costly synthetic compounds are also unaffordable by poor rural farmers.

Thus, identification of a natural, environment friendly substitute of these synthetic chemical compounds can have multifaced application with social benefit. So, the present experiment entitled **"Evaluation of bioactive compounds from selected medicinal plant extracts as root promoter potential"** was conducted in search of any medicinal plant having similar effect as that of growth hormone which can be used and further multiplied.

Ten different medicinal plants are selected which are easily available in our surrounding environment. So, it has been selected *Moringa oleifera, Nyctanthes, Mimosa pudica, Piper betle, Tinospora, Plumbago, Typhonium, Ocimum, Ayapana* and *Azadirachta indica* leaves as medicinal plants. For further analysis the crude extract of plant samples was obtained through Soxhlet apparatus. For soxhlation we need a

suitable solvent in which all medicinal plants can be extracted. That suitable solvent was selected through trail method checking solubility in different medium. In which methanol was found to be most appropriate solvent through which soxhlation was conducted.

The crude extract obtained were further concentrated trough drying and collecting the crude sedimented powder, which was again diluted to make stock solution of 4000ppm. For effective comparison growth hormone IAA and IBA was taken as standard. Fresh semihard wood cutting of *Bougainvillea* was collected and treated for 24 hr and 48 hrs duration in the medicinal plant extract and growth hormones of similar concentration (4000 ppm, 3000ppm, 2000ppm and 1000ppm) in three replications. The treated cuttings were planted in potted mixture contain sand, soil and vermicompost (1:1:1) ration. Observations were recorded after sprouting. It was found sprouting was obtained in many stem cuttings treated with medicinal plant extract. However, the time taken for sprouting in case of *Moringa* and *Piper betle* was minimum i.e. 15-17 days which is comparable to that of growth hormone. Sprouting obtained in other stem cuttings took almost similar time as taken by stem cutting without treatment i.e. almost 30-32 days. Thus, had no special effect as promoter. From further analysis of percentage of sprouting, duration taken for emergence it was found that *Moringa* and *Piper betle* leaf extract has similar effect as that of growth hormone. Abdul *et al (*2020) reported the similar result taking some of the medicinal plants such as Garlic, Black cumin seeds, Liquor ice roost and Egyptian cassia flowers plant extract as root promoting substance in rooting, of semi hard wood cuttings of *Olive*, *Punica*, *Myrtus* and *Ficus*.

Along with this another conclusion was drawn i.e. extending the period of application shows negative effect by retarding growth and percentage of sprouting in both case of growth hormone treatment and medicinal plants extract treatment. Thus, it can be stated that excess application in higher concentration shows toxic effect by slowing down metabolic activity.

Further biochemical analysis was also done to specify the growth promoting activity of medicinal plant extract. HPTLC was performed to check the extent of similarity between standard growth hormone and medicinal plants extract. As we had ten different types of medicinal plant again a small pilot experiment was conducted to

choose suitable mobile phase for HPTLC analysis. For which TLC was conducted taking combination of solvent as mobile phase. From that experiment ethyl acetate: methanol: water at 40:6:4 ratio was taken for further analysis.

HPTLC was conducted taking both growth hormone and medicinal plant extract in a silica coated aluminium plate at 1.5 cm distance as stationary phase and selected solvent as mobile phase. Result was concluded analysing the graph i.e. the Rf range, the peak obtained, the area coverage of the peak and percentage content at that peak. The chromatographic phase was analysed at 254nm and 366nm and also before derivation through spraying and after derivation. Thus, total four different graph was obtained which was cross analysed to get the perfect result. At last, it was concluded that only *Piper betle* and *Moringa oleifera* are having similar peak range and Rf value with maximum area coverage which shows that they can be used as growth hormone substitute.

More specific analysis was done through FTIR analysis by deriving the specific organic compound and functional group present in the medicinal plant extract. The results showed significant response which similar to the chemical constituents thus confirming the auxin like activity of the extract of *Moringa oleifera* and *Piper betle.*

SUMMARY AND CONCLUSION

Medicinal plants have been used from the Vedic era. Traditionally, medicinal plant sector has obtained a significant position in socio cultural life along with health, nutrition and medicinal requirement of tribal and rural population. In past few decades, man being more health conscious traditional herbal medicines is again gaining their importance with lesser side effects. Ornamental flowering plants are associated with mankind since the dawn of civilization. These are one of the wonderful creations of nature. In general, most of the perennial ornamental plant species are multiplied and propagated by the use of various propagation techniques. Stem cuttings have been used for propagation of most of the ornamental plants. However, excess application of plant growth regulators sometimes creates toxicity and also inhibits the rooting. Plant growers are also usually unable to afford those costly chemicals. Medicinal plants are growing in the nature having secondary active compounds with root promoting activity. The present study is to identify the root promoting activity by using ten different medicinal plant extracts and also compared with known plant growth regulators. Stem cutting of *Bougainvillea glabra* were treated with these medicinal plants extract and observation was recorded particularly the growth activity of root induction and its regulation. It was observed that the leaf extract of *Moringa oleifera* and *Piper betle* showed significant effect on shoot & root proliferation within 2 weeks of transplanting in the potted mixture. The percentage of rooting, root growth, number of lateral root formation were significantly higher in treatment with plant extracts obtained from *Moringa oleifera* and *Piper betle* as compared to other plant species. Further, all selected plant extracts had been analyzed through TLC and HPTLC. It was observed that the chemo-profiling of *Moringa oleifera* and *Piper betle* were showing close resemblance with known growth regulators such as IBA and IAA. Further, it had been tested through FTIR to characterize the specific chemical constituents. From the results obtained through FTIR, it was noted that the chemical nature of the active compounds present in the leaf extracts of *Moringa oleifera* and *Piper betle* were similar with IBA and act as auxin like substances. Further biochemical and molecular analysis have not been carried out due to lockdown of the university during COVID 19 pandemic situation from April to July,2021.

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