Discussion

5. DISCUSSION

Orchids, which are grown in different environment conditions and they show highly specialized morphological, structural and physiological characteristics (Dressler, 1990). Mostly Terrestrial orchids grow on the ground surfaces where enough moisture and shade are available and most of them generally seen during the monsoon times. Usually, the tubers of orchids are used in medicinal purposes. Many of the orchids faces threatening issues due to maximum usage and surrounding depletions. They need a special kind of environment and habitat. To work out the the conservation plan for a specific area and to understand the ecology of the species, studies on quantitative information plays an important role (Uniyal et al., 2002).

Now a days Orchids are coming under classification of threatening species. These are becoming a rarity, losing due to selfishness of humans. Orchids are not only important for their aesthetic value but also because they work as ecological indicators. If they vanishes, it indicates a change in the quality of soil and air of the region (Joshi et al., 2009) Hence, In this present work, two orchids namely *Pholidota pallida Lindl* and *Arundina graminifolia D.Don Hochr* which are Endemic to western ghats were chosen to conserve via In vitro regeneration methods and also analysed for their phytochemical activities, antioxidant activity, antimicrobial and anticancer activities, Endophytic fungi isolation , Nanoparticle synthesis activity.

In vitro Regeneration

In vitro propogation is one of the best conservative method so far. The *In vitro* plant propagation has not only important contribution in the knowledge of simple research, but it also proposes budding applications as it assuring suitable for economic purpose industries of plant-derived products (Kumari *et al.,* 2021; Chandran *et al.,* 2020).

Tissue culture plays a important role in vector-independent gene-delivery into plant genome for the production of transgenic plants with improved characterstics. (Hussain *et al.,* 2012; Gleba *et al.,* 2014; Hasnain *et al.,* 2020).

Asymbiotic seed germination

Ongoing loss of orchids due to human invasion to habitats leads to the urge of conserving it. The main obstruction in the production of native orchid seedlings for use in conservation are: (1) progression of effective and steady seed germination methodologies and (2) considering of early seedling growth and development. (Scott L. Stewart et al; 2006). In present study Immature pods were used for the germination and significant growth were analysed after 9 and 12 weeks in KC and BM media for Pholidota and Arundina respectively. Murashige & Skoog (MS), Knudson C medium , and Woody Plant Medium (WPM) culture media were tested for Asymbiotic seed germination and plantlet development for 16 weeks. Tsi is a rare and endangered epiphytic orchid which is endemic to China. This orchid reached the level of extinction due to environment depletion. An effective propagative method was used to conserve this. Seed germination has attained 64% on Knudson's C (KC) medium containing 1.0 mg·L naphthaleneacetic acid (NAA), 10% coconut water, and 1% activated charcoal., 84% of callus formed into protocorm like bodies PLB's from the tenth sub-culture on KC media supplemented with 1.0 $mg \cdot L^{-1}$ NAA, 5% coconut water, and 0.1% activated charcoal formed. This study showed that the protocol is very much efficient means for the large-scale propagation of this endangered orchid. (Song jun zeng; 2011).

Callus Induction

In the Present study, two type of calluses which are pale yellow in colour and in translucent respectively was noticed from 80 days old protocorms. Callus induction was efficient and different in KC and BM media which was in different concentrations and combinations of kinetin, BA and 2,4-D. Addition of these two kinetin and BA affected the callus induction. KC medium with low 2,4 D showed the best callus production.

Likewise, Dimorphorchis lowii is a popular ornamental orchid which is rare epiphytic one endemic to Borneo.For the need of bulk propogation, procedures for callus *In vitro* propagation were developed. The aim of the study was to determine the reactions of plant growth regulators (PGRs) on callus formation from leaf tip explant and the reaction of complex additives on PLBs germination and shoot growth. The best shoot

multiplication from PLBs was noticed in Knudson C (KC) medium added with 15 % (v/v) coconut water. In this treatment, 10.2 ± 6.2 shoots were produced from one callus explant. (Juddy jainol et al; 2017)

In the research, *Cymbidium mastersii* is distributed in Northeast India and also an epiphytic orchid. Due to its high economical value in the horticultural industry, its community are under threat from over-exploitation. Sadly, conventional method of propagation is slow and troublesome, suggesting *In vitro* methods for mass propagation may be more accurate. Four nutrient rich media was used for seed germination and protocorm development: Murashige and Skoog (MS), half-strength MS, Knudson C (KC), and Vacin and Went (VW). In addition, the effects of plant growth regulators 6-benzylaminopurine (BAP), kinetin (KN), α-naphthalene acetic acid (NAA) and indole-3-butyric acid (IBA) was studied in combination and without combination. The highest seed germination percentage was obtained in MS basal medium after 8 weeks of culture. Secondary protocorms were developed from primary protocorms on MS medium nutrient rich with different concentrations and combinations of cytokinins (BAP and KN) and auxins (NAA and IBA). The percentage and germination of seed differ with the composition of the media and was highest in full-strength MS basal medium. (Padmaja Mohanty et al; 2012).

Multiple shoot development

In the current study, regenerative ability of protocorm explants was examined for *P.pallida* and *A.graminifolia*. Protocorms which are produced from *in vitro* cultures of seeds, used as explants reacted quickly on KC (*P.pallida*) and BM (*A.graminifolia*) medium supplemented with growth regulators with various concentrations. Addition of various concentrations of BA and Kinetin was necessary for the plantlet formation from protocorm explants indicated by important changes in the morphogenetic characters of plantlets. In the different combinations used, Kinetin and BA considerably produced multiple shoots from single protocorm explants of *P.pallida* whereas Kinetin produced multiple shoots with moderate number of shoots in *A.graminifolia.*

An In vitro development of plant methodology was successfully established for Cymbidium bicolor an epiphytic orchid by seed culturing from their green pods. Those Immatured seeds were germinated on four basal media viz., Murashige and Skoog (MS) medium, Knudson C (KC) orchid medium, Knudson C modified Morel (KCM) medium and Lindemann orchid (LO) medium. Germination of seed and protocorm was significantly higher in LO medium (96.6 %) followed by KCM (89 %), MS (77.5 %) and KC (62.7 %) media after 56 days. For multiple shoot induction the protocorms were shifted to B5 medium treated with cytokinin. Among the different cytokinins screened, BAP (4.42 μM) produced maximum number of multiple shoots per explant. (Mahendran et al; 2013)

Pseudobulb culture

This study shows that regenerative capacity of pseudobulb's which is of one year old of *P.pallida* and *A.graminifolia*, when screened on both KC and BM medium and with growth regulators. From basal medium, nealy 85 % of explants responded after 40 days, developing a single shoot in *P.pallida* and 90 % responded after 55 days in *A.graminifolia* respectively. In Pholidota the pseudobulb regeneration rate was high as in compared to Arundina.

Similarly, An excellent method for micropropagation of Dendrobium transparens L. using the axenic pseudobulb segments, derived from in vitro germinated seedlings, was developed using half strength of MS basal medium (Sunitibala et al; 2009)

Dendrobium palpebrae Lindl. Which is an rare orchid species of Bangladesh. By growing In vitro plantlets, upper and lower part of pseudobulb segments were especially developed on MS medium enriched with auxins such as IAA, IBA, NAA, Picloram and cytokinins such as BAP, Kinetin. pseudobulb segments regenerated by In vitro both upper and lower part directly produced multiple shoot buds through organogenesis. (Tapash kumar bowmik et al; 2020)

Rooting and Hardening

In this study findings, Rooting was high in Pholidota by the effect of NAA (10.76 µM) while IAA initiated rooting in Arundina (11.4 µM). Fully rooted plants are transferred to pots containing suitable potting mixture with charcoal, bricks, coconut husks, fibres etc.

The current study established a methodology for the speed growth In vitro micropropagation of a threatened and economically valuable most important orchid, Dendrobium primulinum Lindl. Through the shoot tip explant culture (0.3 to 0.5mm) developed from In vitro generated seedlings. The shoot tip explants cultured on Murashige and Skoog (MS) basal medium and MS medium Indivitually or supplemented with combination of different concentrations of growth hormones such as α-naphthalene acetic acid (NAA) and 6-benzylaminopurine (BAP), initiated shoots. The best rooting response was reported on MS medium with supplementation of IAA 0.5 mgl^{-1}. The Fully grown In vitro rooted plantlets were hardened successfully in the potting mixture containing cocopeat and sphagnum moss in the ratio of 2:1. Nearly 70% of plantlets survived in this study. (Bijaya pant et al; 2012).

Phytochemical analysis and biological evaluation

Preliminary phytochemical analysis

In Preliminary phytochemical analysis of *P.pallida* in this study showed the presence of alkaloids, carbohydrates, glycosides, saponins, terpenoids, steroids, flavonoids, phenolic compounds, proteins, gum and mucilages, phytosterols, tannins and phlobatannins, and it gave negative outcomes for amino acids, anthroquinones, fats and oils and carotenoids. chloroform extract revealed the presence of high phytochemical components followed by water extract. Preliminary phytochemical test of *A.graminifolia* shows the presence of alkaloids, carbohydrates, glycosides, saponins, terpenoids, steroids, flavonoids, phenolic compounds, proteins, phytosterols, fats and oils, tannins and phlobatannins, it gave negative out comes to protein, amino acids, anthroquinones, gum and mucilages, and carotenoids. The presence of phytochemicals in the leaf extracts of both *P.pallida* and *A.graminifolia* proved their pharmacological importance.

In another study, the Physicochemical confines showed the presence of bioactive compounds. The phytochemical testing detected the presence of Alkaloids, Tannins, Flavonoids, Sopanins, Steroid and Terpenoids. (Keerthiga et al; 2014)

On the other hand, Phytochemical screening revealed the presence of alkaloids, carbohydrates/glycosides, flavonoids and unsaturated sterols and triterpenes in Satyrium nepalense orchid. (Abhay Prakash Mishra et al; 2012)

Total phenol and flavonoid content

In this current study, chloroform extract of *P.pallida* showed highest phenolic content (86.55 ± 0.84 µg) followed by aqueous extract with a value of 69.63 ± 0.54 µg GAE/ mg DW. The methanolic extract of *A.graminifolia* showed highest phenolic content (75.85 ± 0.47 µg) followed by aqueous extract. The total flavonoid content of chloroform extract of *P.pallida* was detected to be higher with the value of 58.60 µg QE/mg DW than other extracts. Methanol extract of *A.graminifolia* detected the maximum value of 59.86 µg QE/mg DW.

Maximum polar compounds such as phenolic and flavonoid substances are strong prohibitors of reactive oxygen species attack (Owen *et al*., 2003). The biological properties, consisting of cytotoxic and antioxidant properties, of flavonoids are considered in an test of the medicinal and nutritional values (Harborne and Williams, 2000). The study on Phalaenopsis orchid was conducted to determine the total phenolics, total flavonoids, and antioxidant activity of ethanol extracts from the leaves and roots of six commercial hybrid species. Foliar extracts of "Chian Xen Queen" contained the highest total phenolics with a value of 11.52 ± 0.43 mg gallic acid equivalent per g dry weight and the highest total flavonoids (4.98 ± 0.27 mg rutin equivalent per g dry weight). (Truong Ngoc Minh et al; 2016).

Fourier Transform Infrared Spectroscopy (FT-IR) spectrum analysis

FTIR study was conducted on leaf extracts of both orchids ie; Pholidota and Arundina and gained peak area of 3398.57cm and 3429.43 cm on Infrared spectrum in extracts of water and chloroform. IR spectrum of aqueous and methanol extract of

A.graminifolia shows peak area 3428.44cm^{-1},3378.20 $cm^{-1}$and 2728.34 cm^{-1} in the aqueous and methanol extract of *A.graminifolia.* There was no absorbance in between the region 2220-2260 cm^{-1} in *P.pallida* and *A.graminifolia* which shows that there was no cyanide group in the leaf extracts. This result shows that the plants does not contain any toxic substances.

Fourier transform infrared (FTIR) is an simple, quick, low-cost, and one of the high sensitivity spectroscopic method. As fingerprints of each and every person, the Infrared spectrum of any matter is known to be specific, which allows Infrared spectroscopy to be applied to identify unknown samples or classify different samples. FTIR spectroscopy has been used in many classificational studies. So, it is an efficient way to identify the type of orchids with similar appearance. (Yufeng chen et al; 2019)

Similarly, The GM content of tubers of 14 varities of orchid species was examined and compared using Fourier transform infrared (FTIR) spectroscopy and an enzymatic colorimetric method. The results gained from the analyzed modes, the sum of the peak areas at 873 and 812 cm^{-1}, which represent the CH bending attributed to the β-pyranose form of D-glucose and D-mannose, respectively. (Arda acemi et al; 2019)

Gas Chromatography - Mass Spectrometry (GC-MS) in phyto chemical analysis

In the current study, the GC-MS analysis of *P.pallida* leaves detected the presence with eight compounds along with major and minor constituents that could enrich to the medicinal quality of the plant. The major components were Naphthalene (40.04 %), Ergost-5-en-3-ol (25.26 %), Phenol, 4-methoxymethyl- (16.40 %), n-Hexadecanoic acid (6.01 %) and minor components were 1-Heptanol, 6-methyl- (3.92 %), trans-Cinnamic acid (3.19 %), Phosphonic acid, dioctadecyl ester (2.99 %) and 1-Heptanol, 3-methyl- (2.20 %).

The GC-MS analysis of *A.graminifolia* leaves showed the presence of five compounds along with major and minor compounds such as 4H-1-benzopyran-4- one, 5,7-dihydroxy-2-phenyl- (84.74%), 4H-1-benzopyran-4-one, 2,3-dihydro-5,7- dihydroxy-2-phenyl- (14.26 %), Pentadecanoic acid (0.37%), Butane-1,2,3,4-tetraol (0.32 %) and Dibenzyl sulfide (0.31 %).

Hexadenoic acid reported in previous times as a constituent in alcohol extract of the leaves of *Kigelia pinnata* (Grace *et al*., 2002) and *Melissa officinalis* (Sharafzadeh *et al*., 2011). Parasuraman *et al*. (2009) detected 17 compounds with n-Hexadecanoic acid and Octadecanoic acid as the major constituent in the leaves of *Cleistanthus collinus.* Devi *et al*. (2009) concluded that leaves of *Euphorbia longan* mainly possess n-hexadecanoic acid and 9, 12-Octadecadienoic acid. These reports are correlates with the result of the present study.

Antimicrobial activity

Antibacterial screening disc diffusion method

The extracts of Pholidota and Arundina tested for their antimicrobial activity. Out of which seven bacteria was screened, ethanolic leaf extract of *P.pallida* inhibited the growth of three Gram-positive bacteria and two gram negative bacteria, Maximum zone of inhibition was obtained with the *P. aeruginosa.* In the case of *A.graminifolia* tested for antibacterial activity, against the methanol and chloroform extracts exhibited maximum inhibition.

The research results are analogous to the reports of Uma *et al*. (2009) and Shanmugavalli *et al.* (2009) in *D. nobile, Bletilla striata* and in *Vanilla planifolia*.

Minimum Inhibitory concentration

The present study shows, the MIC of the chloroform extract of *P. pallida* was 0.410 mg/ml against *E. faecalis*, MIC of the methanol extract from *A. graminifolia* leaves was 0.360 mg/ml against *E. faecalis.* The medium antibacterial activity was identified against *S. aureus* and *S. enterica* with the MIC value 0.650 mg/ml and 0.600 mg/ml.

Research proves that the strong antimicrobial activity highly related on MIC values, which ranges between 0.05 - 0.50 mg/ml, moderate activity on values between 0.6 and 1.50 mg/ml and weak activity in the range above 1.50 mg/ml (Gupta and Saxena, 1984). The current results proves that the chloroform extract of *P.pallida* leaves and methanol extract of *A.graminifolia* leaves possess strong antibacterial activity.

Antioxidant activity

DPPH radical scavenging assay

In the current study the observed findings is that the DPPH scavenging activity in aqueous extract of *P.pallida* leaves with IC_{50} value 127 μg/ml, and (DPPH) scavenging activity of *A.graminifolia* was noted in the aqueous extract with IC_{50} value of 318.0 μg/ml.

The free radical scavenging activity by DPPH assay in different orchids and proved that, 40 ppm flavidin and BHA exhibited 95.6 % and 93.3 % free radical scavenging activity by DPPH method (Guddadarangavvanahally et al; 2004). Onthe other hand, orchids such as *Anoectochilus formosanus, A. roxburghii, Dendrobium amoenum, D. nobile, D. tosaense, D. moniliforme,* and *Gastrodia elata* shows antioxidant activity (Lo *et al*., 2004b; Zhang *et al*., 2007; Gutierrez, 2010). The constituents such as ephemeranthone isolated from *Ephemerantha Ionchophylla* and several phenanthrenes, phoyunnanins A- C, 9,10-dihydrophenanthrene, 4,4',7,7'-tetrahydroxy-2,2'-dimethoxy-9,9',10, 10'- tetrahydro-1,1'- biphenanthrene, lusianthridin, eulophiol, 2,4,7-trihydroxy-9,10- dihydrophenanthrene and imbricatin has been obtained from 60% of ethanol extract of *Pholidota yunnanensis,* which possess higher antioxidant activity (Chen *et al*., 1999; Guo *et al*., 2007).

Antioxidant activity was identified by 2, 2-diphenyl-1-picryl hydrazyl (DPPH) free radical scavenging analysis and defined as half inhibition concentrations (IC_{50} values). Antioxidant activity was maximum in the stems of Vanda cristata and least in the leaves of G. acutifolius. Some of the orchid extracts tested for antioxidant activity which were comparable to or even higher than those found for extracts of medicinal plants studied previously. (Mukesh babu chand et al; 2016).

Reducing power assay

In this study, the reducing power assay showed the aqueous leaf extract of both *P.pallida* and *A.graminifolia* has higher antioxidant activity.

In the reducing power assay, the flower extract of the white orchid revealed the most capable extract, followed by the leaf extract of the yellow orchid and the flower

extract of the purple orchid. Colour of the flower and antioxidant activity relation of these orchids showed them to be potential sources of antioxidants for both pharmaceutical use and stress-tolerance in these orchids (Hoang chinh ngyuen et al; 2018).

FRAP Assay

In this work the range of ferrous reducing capability of different extracts of *P.pallida* leaves were varies from 130.59–499.65μM of AAE/g DW whereas in the case of Arundina the ferrous reducing ability varies from 36.75–227.74 μM of AAE/g DW

The FRAP assay (ferric reducing ability of plasma) tested total antioxidant power and was chosen to determine the possible effects of medicinal plants (Szollosi and Varga, 2002). FRAP assay depends upon the ferric tripyridyltriazine (Fe (III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe (II)-TPTZ) by a reductant at low pH. Fe (II)-TPTZ has an deep blue colour and can be screened at 593 nm (Benzie and Strain, 1996).

Anticancer Activity

Cytotoxic activity in Pholidota and Arundina leaf extracts has been tested based on the antioxidant activity. The extract was investigated using MTT assay on human breast cancer cell line. Pholidota leaf extract was very much active in inhibiting the MCF-7 cell growth comparing to Arundina. The IC50 value obtained for *Pholidota pallida* on MCF-7 cell line was 170.3μg/ml and that for Arundina was 95.5μg/ml.

The screening of the phytochemical constituents and anticancer activities in foliar extract of Aerides odorata Lour., an Orchid, revealed In vitro anticancer activity by using against two cancer cell lines (MCF-7 and HeLa cell line) by using MTT assay. Among two extracts a total 13 constituents possess anticancer activity. The solvent extracts possess significant cancer cell growth inhibition with IC50 value ranging between 26.211 μg/mL to 59.061 μg/mL. (Jhansi katta et al; 2019).

Nanoparticle synthesis

In this study, Aqueous extracts of Pholidota and Arundina were chosen for the synthesise of nanoparticles. Antioxidant activity of silver nanoparticles were analysed by

Total antioxidant assay and FRAP assay. Where Total antioxidant assay showed more activity than FRAP assay of 503 mg/gm and 508 mg/gm for Pholidota and Arundina respectively. Antimicrobial activity of nanoparticles were also calculated using E.coli and S.aureus for anti-bacterial and A.flavus and Fusarium sps for anti-fungal activity. E.coli and A.flavus possess good activity against nanoparticles 7mm each. For determining the morphological analysis Scanning Electron Microscopy (SEM) and X-Ray diffraction method (XRD) analysis also tested.

Synthesis of silver nanoparticles that are familiar mainly for their antimicrobial activity can be achieved through various methods. As the most established nanoparticles are synthesized using plant extracts, the antioxidant capacity of AgNPs is often compared with plant extract itself. The results are conflicting, as some authors noticed higher antioxidant ability of silver nanoparticle's while on the other hand, there are also tests with the opposite results. (Zdenka Bedlovicova et al; 2020).

Vanda tessellata is an orchid which is used so many years for treating various diseases. The aqueous extract of V. tessellata leaves are used for silver nanoparticle synthesis. Nanoparticles were characterized using Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction (XRD), Scanning Electron Microscopy (SEM) and UV–Vis spectroscopy. The antibacterial ability of the synthesized silver nanoparticles was screened against pathogenic bacteria such as Salmonella sps, Staphylococcus aureus and Escherichia coli. The AgNPs synthesized were 15nm with size range 10 to 50nm with cubic and hexagonal shape. Antioxidant activity of AgNPs was tested by DPPH method. AgNPs showed very much antioxidant and antibacterial activity. Green synthesis of AgNPs with efficient antioxidant and antibacterial activity may help in the development of budjet friendly therapeutic agents. (Manjunath hulikare; 2014).

Isolation, identification and bioactivity evaluation of endophytes

Isolation and identification of endophytes

In the study, Endophytes were developed from leaf ends of the Pholidota and Arundina. The DNA isolation were carried out by Agarose Gel electrophoresis and amplification of DNA were done using primers ITS1 and ITS4. The endophyte which is

isolated from *A. graminifolia* leaf confirmed its taxonomic affinity (as on BLAST n search, CLUSTAL W software and Gen bank) to *Colletotrichum karstii*.

Bayman and Otero (2006) enlisted so many diverse forms of fungal endophytes species which are isolated from epiphytic, terrestrial and lithophytic orchids. So many research had proved about the diversity of endophytic fungal communities, consisting of mycorrhizal and non-mycorrhizal fungi in tropical orchid plants (Bayman *et al*., 1997; McCormick *et al*., 2004) .

The current study states the isolation of non-mycorrhizal. Orchids are always clingy on mychorrhizal fungi for nutrition, growth and development after seed germination (Smith and Read, 1997; Bonnardeaux *et al*., 2007). The commonly seen non-mycorrhizal fungal are *Acremonium* spp., *Fusarium* spp., *Trichoderma* sps., *Rhizoctonia* spp. and *Alternaria* spp., and most of these fungi comes under endophytes (Bayman and Otero, 2006; Chang, 2007).

In some plants, the presence of one endophyte has been shown to decrease or lessen plant infection by other fungi. In two species of grass plant, endophytic plant had lower levels of leaf diseases than endophyte-free plants (Clay, 1991). In *Cyperus rotundus* leaves contain either *Rhizoctonia solani* or the epiphyte *Balansia cyperi*, but not both (Stovall and Clay, 1991). Tao *et al*. (2008) stated that only one mycorrhizal species (*Sebacina* sp.) within roots, and this was the leading one species as found within *Caladenia carnea* (Bougoure *et al*., 2005). This is analogous to our study that the one endophytic fungus was present in each orchid leaf (S1 in *P.pallida* leaf and S2 in *A.graminifolia* leaf).

Past experimental studies on *Guignardia* and *Phyl-losticta* have picturize the bewilderment that still exists surrounding species concepts in literature. For an example, both *Guignardia endophyllicola* and *G. mangiferae* have been connected to *P. capi-talensis* as anamorph (Okane *et al*., 2001). This problem was settled by Baayen *et al*. (2002), who successfully used ITS DNA sequence data to show that *G. endophyllicola* was not specific with *G. mangiferae*. ITS region sequencing is the most common and strong method for the identification of orchid endophytic fungi (Taylor and Bruns, 1999;

Kristiansen *et al*., 2001; Sharon *et al*., 2008). Molecular analysis of Internal Transcribed Sequences (ITS) stated the existence of sequences of *Tetracladium* in roots of an endangered orchid *Orchis militaris* in Northern Italy (Elena *et al*., 2010). Whereas the sequences of *Tetracladium* were also seen in roots of *Cephalanthera longifolia* of Estonia (Abadie *et al*., 2006).

Evaluation of antibacterial and antioxidant activity of the isolated endophytes

All the extracts (S1- ethanol, distilled water, spent broth-ethanol S2- ethyl acetate, distilled water, spent broth-ethyl acetate) from both the orchids, was not effective in controlling the growth of the seven bacteria (*P. aeruginosa, E. facealis, E.coli, S. aureus, B. subtilis, S. enteria* and *C. diphtheriae*) used. The extracts (S1, S2 and spent broth) did not contain antioxidant properties.

In match to the current research study many statements that supported the ability antimicrobial activity of endophytes are available. Out of 24 isolates gained from *Garcinia mangostana,* 10 isolates could prohibit some human pathogenic bacteria which are examined (Radji *et al*., 2011). The culture broth ethyl acetate extract of *Guignardia* was active against *S. aureus* and *E. coli* (Rodrigues *et al*., 2000). Suzete *et al*. (2011) results states that *Epicoccum nigrum* extract and *G. vaccinii* have capability to prohibit *Diatraea saccharalis* larvae development and they demonstrated to be potent for a future use in biological control of sugarcane borer. Furthermore, a novel indole derivative from endophytic fungus of *Colletotrichum* sp. Revealed slightly medium antibacterial activity against Gram-positive bacteria of *B. subtilis, S. aureus, Sarcina lutea* and *Pseudomonas* (Tan and Zou, 2001).