DISCUSSION

Plants contain accumulated natural products, biologically active materials and ingredients which have various effects. Plant derived dietary supplements, phytochemicals and pro-vitamins give a hand in maintaining good health and combating diseases. The need for more potent, safe and affordable drugs has led to intensified research on herbal drugs, the result of which is the introduction of new herbal preparations for therapeutic uses. Many phytochemicals are known to combat oxidative stress in the human body by helping to maintain a balance between oxidants and antioxidants. The role of plants in prevention or control of disease has been attributed to antioxidant properties of their constituents.

Parasitic plants are diverse, present in all major ecosystems and have strong impact on community structure and dynamics. While parasites have strong negative impact on their hosts, different hosts can also have an effect on their parasites by both direct and indirect ways. Because, parasitic plants acquire both nutrients and defensive compounds from their hosts and this may be the attributes of the parasitic plants.

The mistletoes constitute a polyphyletic group of perennial flowering parasitic plants attached to branches of other trees and shrubs as obligate aerial stem parasites. Several species of *Loranthus* (belongs to Loranthaceae -Showy Mistletoe Family) play a vital role in natural plant communities by interacting with other hosts, herbivores and dispersers. The mistletoe species not only adorn, festive and portrait of friendship, but also are still respected palliative for the most feared disease including
cancer, with their connotations in sympathetic medicines of abnormal growth. The chemical constituents and biological activities of the plants depend to a large extent on the host plant and the season of the harvest (Tuquet and Salle, 1966; Mortan, 1977).

Several earlier reports revealed that the hemiparasitic Loranthus species are known to produce variety of bioactive compounds. Information on the mistletoes of different countries (European, Australian and Korean) and the differences between the metabolic pathway, characteristics of phytochemicals and bioactive properties have been documented by many workers (Kuttan et al., 1990; Joller et al., 1996; Fernandez et al., 1998; Yoon et al., 1998). The results of the present study on phytochemical analysis and bioactivities of Dendrophthoe falcata plant (leaf bark and tender shoot) samples collected from Artocarpus heterophylla host tree are discussed hereunder.

The hemiparasite, Dendrophthoe falcata collected from Artocarpus heterophyllus host tree, during October, 2012 around Marthandom town, Kanniyakumari district shows similar morphometric features as described in Gamble flora and also identified with the BSI at Coimbatore. But there are some variations noted in the leaf length, leaf breadth, and flower length in Dendrophthoe falcata collected from A. heterophylla host tree which may be due to the nature and type of nutrients received by the hemiparasite from the host tree.

Most parasitic plants can potentially attack a large number of different co-occurring species (i.e., they have a broad host range), often simultaneously (Gibson and Watkinson, 1989; Nilsson and Svensson, 1997; Pennings and Callaway, 2002; Westbury, 2004). In this respect, most parasitic plants can be considered as
generalists. Examples of wide host range are documented for shoot parasites. Among
the parasitic plants, the tropical rain forest mistletoe, *Dendrophthoe falcata*, is a
climbing woody parasitic plant (Dashora et al., 2011). It is indigenous to tropical
regions, especially India, Sri Lanka, Thailand, China, Australia, Bangladesh, Malaysia
and Myanmar. In India, it is widely distributed throughout up to 900 m (Pattanayak et
al., 2008). It has approaching 400 known host species (Narasimha and Rabindranath,
till date, show that parasites often prefer or perform better on host with high nitrogen
content such as legumes (Schulze and Ehleringer, 1984; Kelly, 1992; Seel and Press,
1996; Matthies, 1995), or host that have readily accessible vascular systems (Kelly
et al., 1988) and/or lower defense capacity (Cameron, 2004; Cameron et al., 2005).

According to Scott (1817) and Bidie (1874), plants having deciduous bark
with dry membranous layers are unfavourable hosts of *Loranthus* parasite. However,
Fischer (1926) dissents from the above view and has mentioned that a number of
plants have been attacked regularly by the parasite, which have similar characters of
the bark, but very few plants of the family Myrtaceae, especially the non-indigenous
genera, are recorded by him as host plants (Srivastava, 1935). However, Hawksworth
et al. (1993) listed 401 hosts among 227 genera and 77 plant families for
*Dendrophthoe falcata*. There are more than 400 plants for the species of *Loranthus*
reported. Chandrakasan (2013) reported that the hemiparasite, *L. longiflorus*
(*Dendrophthoe falcata*), generally considered to have a broadest host range by
inhabiting about 39 host tree species, belongs to 37 genera and 23 families around
Nagercoil town, Kanniyakumari District. In this study, it was noted that the
hemiparasite *Dendrophthoe falcata* parasitize about 31 species belongs to 29 genera
and 19 families from the study area of Mardhandam, Kanniyakumari District, Tamil Nadu (Table 1). The leaf, tender shoot and bark samples of *Dendrophthoe falcata* were collected from *Artocarpus heterophylla* host tree. The impact of the host tree on the phytocomponents and bioactive properties of *Dendrophthoe falcata* is evaluated and compared.

Extractive value of different solvent extracts help to determine the amount of active constituents in a given amount of medicinal plant material when extracted with solvents. These values provide an indication of the extent of polar, medium polar and non-polar components present in the plant material. It is employed for those plant materials for which no suitable or biological assay method exists. In this study, the extractive value of different solvents are determined in the leaf, bark and tender shoot samples of *Dendrophthoe falcata* collected from *Artocarpus heterophyllus* host tree and it is noted that among the extracts tested aqueous extract shows maximum extractive value in the leaf, bark and tender shoot samples. Among the *Dendrophthoe falcata* samples, tested, the leaf sample shows maximum extractive value than the bark and tender shoot samples (Table 3). The low extractive yield of solvent extracts may be due to the low solubility of the major components of the aerial parts in solvents as suggested by Pattanayak *et al.* (2011).

Plants produce primary and secondary metabolites which encompass a wide array of functions (Croteau *et al.*, 2000). Primary metabolites, which include amino acids, simple sugars, nucleic acids, and lipids, are compounds that are necessary for cellular processes. Secondary metabolites include compounds produced in response to stress, such as the case when acting as a deterrent against herbivores (Keeling and
Bohlmann, 2006). Plants can manufacture many different types of secondary metabolites, which have been subsequently exploited by human beings for their beneficial role in a diverse array of applications (Balandrin et al., 1985). Often, plant secondary metabolites may be referred to as plant natural products, in which case they have illicit effects on other organisms. Preliminary phytochemical screening of plant is very useful for the determination of the active constituents in different solvent extracts (yields). Dashora et al. (2011) revealed the presence of carbohydrates, phytosterols, flavonoids, glycosides and phenolic compounds in *Dendrophthoe falcata*. Pattanayak et al. (2011) reported that the chloroform extract of *Dendrophthoe falcata* had showed positive result for steroids, terpenes, flavonoids, while methanol extract had revealed the presence of steroids, tannins, terpenes, glycosides and flavonoids. Most of the active principles are found in alcoholic and aqueous extracts. The results of the present study also are in agreement with the results of previous reports (Tables 7 to 9).

The GC-MS analysis for the active principles in the ethanol extract of *Dendrophthoe falcata* indicated the presence of 12 compounds in the leaf harvested from *Artocarpus heterophyllus* host tree (Table 10), one compound in the tender shoot sample (Table 12) and one compound in bark sample (Table 13). Among the compounds identified, in the ethanol extract of *Dendrophthoe falcata* leaf sample, 1-terpine alcohol, 1-diterpine, 2-triterpines, 1-fatty acid ester, 1-linolenic acid, 1-plasticizer, 2-palmitic acid, 1-vitamin, 2-steroids compounds. Most of these compounds reported to have various bioactivities including antimicrobial, anti-inflammatory, antioxidant, antiandrogenic, anticancer, antiaene, antihistaminic, anticonary, antiezemi, antiarthritic, antifouling, antitumor, antiageing, antidiabetic,
antidermatic, antilukemic, antiulcerogenic, antispasmodic, antibronchitic, antiasthma, antimalarial, antiviral, antihyperglycemic, antiflu, antiangiogeni, 5-alfa-reductase inhibitor, chemopreventive, cytotoxic, diuretic, hemolytic, hepatoprotective, hypochloresterolemic, immunostimulant, immunosuppresent, inhibition of HIV-1 protease, insectifuge, lipoxigenase-inhibitor, lubricant, nemeticide, pesticide and vasodilator properties. No activity was reported in the fatty acid eater compound (Table 11). On the other hand, one compound (plasticizer compound) was identified in the ethanol extract of *Dendrophthoe falcata* bark and tender shoot samples harvested from *Artocarpus heterophyllus* host tree (Tables 12 & 13) which was reported to have antifouling and antimicrobial activities. The biological activities of the phytocompounds identified in the ethanol extract of *Dendrophthoe falcata* leaf, tender shoot and bark samples harvested from *Artocarpus heterophyllus* host tree were based on Dr. Duke’s Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/ USDA. This study helped to identify the compounds present in the leaf, bark and tender shoot samples of *Dendrophthoe falcata* obtained from *Artocarpus heterophyllus* host tree, a hitherto uninvestigated species.

The biochemical content determined in the leaf, tender shoot and bark samples of *Dendrophthoe falcata* collected from *Artocarpus heterophyllus* host trees reveals that the *Dendrophthoe falcata* leaf sample has more amount of starch and less amount of protein, carbohydrate, and reducing sugar content as compared to the tender shoot and bark samples (Table 17). The tender shoot sample of *Dendrophthoe falcata* possesses fewer amounts of all the four compounds tested as compared to leaf and bark samples. The bark sample of *Dendrophthoe falcata* shows high content of
carbohydrate, protein and reducing sugar and less amount of starch content than the leaf and tender shoot samples. This study shows that plant parts having rich primary metabolites can be used for the biosynthesis of secondary metabolites or bioactive compounds. The analysis of minerals in the leaf, tender shoot and bark samples of *Dendrophthoe falcata* collected from *Artocarpus heterophyllus* host tree reveals the presence of calcium, iron, magnesium and phosphorus at varied concentrations while the zinc was not detected or in trace amount (Table 18).

The search for raw materials containing potent antioxidants continues to attract the attention of researchers. Fruits, vegetables, seeds and spices are all known to be rich sources of natural antioxidants, and medicinal plants are another important source for a wide variety of natural antioxidants (Ren-You Gan et al., 2011). The antioxidant property of plant might be due to their phenolic compounds (Cook and Samman, 1996; Motalleb et al., 2005) including tannins and flavonoids and they have been reported as promising antioxidants (Kivitis et al., 1997). Antioxidants act as radical scavengers when added to the food products and prevent the radical chain reaction of oxidation that delay or inhibit the oxidation process and increase shelf-life (Young and Woodside, 2001). In the recent years, interest in the study of antioxidant activity of plant extracts (Azaizeh et al., 2005) and isolation of antioxidants from plants have grown due to the fact that the free radicals have been related to degenerative diseases (Joyeux et al., 1995; Willcox et al., 2004). Angiospermic hemiparasitic plant *L. longiflorus* reported to contain biologically active substances (Ramachandran and Krishnakumary, 1999; Rastogi and Mehotra, 1993; Kacharu and Krishnan, 1979). *Loranthus parasiticus* reported to possess the highest antioxidant capacities and total phenolic content among 50 plants tested, and could be rich potential source of natural antioxidants (Ren-You Gan et al., 2011).
Flavonoids are "the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants" (Spencer and Jeremy, 2008) and are categorized according to chemical structure, into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones. Over 4,000 flavonoids have been identified, many of which occur in fruits, vegetables and beverages (tea, coffee, beer, wine and fruit drinks). Flavonoids are the most important plant pigments for flower colouring. Some flavonoids have inhibitory activity against organisms that cause plant disease. Preliminary research indicates that flavonoids may modify allergens and carcinogens, and so may be biological "response modifiers". The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health and have been reported to have anti-allergic, anti-inflammatory (Yamamoto and Gaynor, 2001), anti-microbial (Cushnie and Lamb, 2005; 2011), anti-cancer (de Sousa et al., 2007), and anti-diarrheal activities (Schuier et al., 2005).

Total flavonoids are group of effective antioxidants which are present abundantly in plants. Flavonoids and their relative compounds are found to exhibit numerous biological activities like vasodilatory, anticarcinogenic, anti-inflammatory, antibacterial, immunostimulating, antiallergic and antiviral effects (Middletone and Kandaswami, 1992) and are effective in scavenging hydroxyl radicals (Lean et al., 1999) and in DPPH radical (Apati et al., 2003). In this study, the qualitative and quantitative analysis of an antioxidant flavonoid compound was carried out. The HPLC analysis of flavonoid compound profile analysis shows the presence of five compounds such as caffeic acid, ferulic acid, gallic acid, quercetin and rutin compounds with varying concentrations in the Dendrophthoe falcata leaf, tender
shoot and bark samples ethanol extracts (Tables 14 to 16). The ethanol extract of *Dendrophthoe falcata* leaf sample was found to possess more amount of rutin flavonoid is followed by caffeic acid, feruleic acid, quercetin and gallic acid. In the tender shoot sample ethanol extract possesses caffeic acid, gallic acid, and rutin in decreasing order of concentration while quercetin and ferulic acid were under below detection limit. The bark sample extract shows more amount of gallic acid while all other flavonoids are only under below detection limit. Flavonoids and their relative compounds are effective in scavenging hydroxyl radicals (Lean *et al.*, 1999) and in DPPH radical (Apati *et al.*, 2003).

The generation of antioxidant species (AOS) is a common event in growth and developmental processes of organisms (Thompson *et al.*, 1987; Paliyath and Droillard, 1992; McKersie and Leshem, 1994). During normal condition, growth and development of plants are invariably exposed to several forms of stress. AOS are commonly generated under the stress conditions (Scandalios, 1993; Allen, 1995; Anderson *et al.*, 1995; Rao *et al.*, 1996). Plants exposed to stress are connected with oxidative damage at the cellular level (Foyer and Noctor, 2003). By virtue of their chemical properties, AOS are highly reactive and have the potential to damage membrane lipids, proteins, chlorophyll, and nucleic acids, thus disrupting the homeostasis of the organism (Shaaltiel and Gressel, 1986; Scandalios, 1993). If there is a serious imbalance in any cell compartment between the production of reactive oxygen species (ROS) and antioxidant defense, oxidative stress and damage occurs (Mittler, 2002). Even under normal growth conditions, low amounts of ROS such as superoxide radical (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), hydroxyl radical (OH), and singlet oxygen (1O$_2$) are produced as metabolic byproducts of plant cells (Cai-Hong
et al., 2005). As a result, plants have developed the scavenging mechanism of ROS categorized as enzymatic and non-enzymatic mechanisms to prevent or alleviate the damage (Reddy et al., 2004; Demiral and Turkan, 2005). These mechanisms include scavenging the AOS by natural antioxidants, and by use of an enzymatic antioxidant system that includes SOD, CAT, POX, APX, and GR, many of which act in tandem (Scandalios, 1993; Foyer et al., 1994; Allen, 1995; Anderson et al., 1995; Rao et al., 1996). In order to scavenge the ROS, SOD produces hydrogen peroxide as reaction product. This hydrogen peroxide in turn is neutralized into H₂O and O₂ by the activity of another antioxidant enzyme peroxidase. When ROS increases, chain reactions starts in which superoxide dismutase (SOD) catalyzes the dismutation of O₂⁻ radicals to molecular O₂ and H₂O₂ (Meloni et al., 2003). The H₂O₂ is then detoxified in the ascorbate–glutathione cycle (Asada, 1999; Mittler, 2002), which involves the oxidation and re-reduction of ascorbate and glutathione through the ascorbate peroxidase (APX) and glutathione reductase (GR) action (Foyer and Halliwell, 1976; Noctor and Foyer, 1998).

Superoxide dismutase (SOD) antioxidant enzyme constitutes the first level of defense against ROS within plant cell. Superoxide is one of the main reactive oxygen species in the cell. Consequently, SOD serves a key antioxidant role. In higher plants, superoxide dismutase enzymes (SODs) act as antioxidants and protect cellular components from being oxidized by reactive oxygen species (ROS) (Alschger et al., 2002). Superoxide is known to denature enzymes, oxidize lipids, and fragment DNA (Smirnoff and Nicholas, 1993). SODs catalyze the production of O₂ and H₂O₂ from superoxide (O₂⁻), which results in less harmful reactants. When get used to increased levels of oxidative stress, SOD concentrations typically increase with the degree of
stress conditions. The compartmentalization of different forms of SOD throughout the plant makes them counteract stress very effectively. SOD has powerful anti-inflammatory activity. Treatment with SOD decreases reactive oxygen species generation and oxidative stress and thus, inhibits endothelial activation and indicates that modulation of factors that govern adhesion molecule expression and leukocyte-endothelial interactions. Therefore, such antioxidants may be important new therapies for the treatment of inflammatory bowel disease (Segui et al., 2004).

Another antioxidant enzyme is catalase, which is involved in the cellular defense system through the elimination of hydrogen peroxide. Catalase is found in living organisms that changes hydrogen peroxide to water and oxygen. Hydrogen peroxide is formed as a toxic waste product of metabolism. It must be quickly converted into other, less dangerous, chemicals. To manage this problem, the enzyme catalase is frequently used to rapidly catalyse the decomposition of hydrogen peroxide into harmless oxygen and water.

Peroxidase is one of many numbers of protein-based enzymes that acts as catalysts to facilitate a variety of biological processes. Specifically, peroxidase activity involves donating electrons to bind to other substrate substances, such as ferrocyanide and ascorbate, in order to break them down into harmless components. Most notably, peroxidase enzymes degrade hydrogen peroxide, a naturally occurring byproduct of oxygen metabolism in the body. As a result, this substance is converted into water and oxygen.

Glutathione reductase (GR) plays an indirect but essential role in the prevention of oxidative damage within the cell by helping to maintain appropriate
levels of intracellular glutathione (GSH). GSH, in conjunction with the enzyme glutathione peroxidase (GP), is the acting reductant responsible for minimizing harmful hydrogen peroxide cellular levels (Meister, 1994). Thus, glutathione reductase reduces glutathione disulfide (GSSG) to sulfhydryl form GSH, which is an important cellular antioxidant (Mannervik, 1987; Meister, 1988). The regeneration of GSH is catalyzed by GR (Andersen, et al., 1997). These studies revealed the importance of various antioxidant enzymes in the protection of human health by scavenging the generated AOS. The antioxidant enzymes analysed in this study, in Dendrophthoe falcata leaf, tender shoot and bark samples, also showed significant catalase, glutathione reductase, peroxidase and super oxide dismutase enzyme activities. The aqueous extracts of Dendrophthoe falcata leaf samples collected from Artocarpus heterophyllus showed more enzyme activity as compared to the tender shoot and bark samples. Among the enzymes tested, catalase enzyme showed maximum activity in the leaf and tender shoot samples while in bark samples the glutathione peroxidase enzyme shows maximum activity (Table 19).

Many synthetic antioxidants reported to have several side effects (Osawa and Namiki, 1981; Ito et al., 1983; Gao et al., 1999; Williams et al., 1999). Therefore, there is a need for more effective, less toxic and cost effective antioxidants. Medicinal plants appear to have these desired comparative advantages, hence the growing interest in natural antioxidants from plants (Rice-Evans, 2004; Pattanayak et al., 2011). Several phytochemical surveys have mentioned that the antioxidant property of plants might be due to their phenolic compounds (Cook and Samman, 1996; Motallab et al., 2005) including tannins and flavonoids and they have been reported as promising antioxidants (Kivitis et al., 1997). Antioxidants act as radical scavengers
when added to the food products and prevent the radical chain reaction of oxidation that delay or inhibit the oxidation process and increase shelf life by retarding the processes of lipid peroxidation (Young and Woodside, 2001). In recent years, interest in the study of antioxidant activity of plant extracts (Azaizeh et al., 2005) and isolation of antioxidants from plants have grown due to the fact that the free radicals have been related to degenerative diseases (Joyeux et al., 1995; Willcox et al., 2004). The antioxidant activities were assessed earlier in the extracts of *L. parasiticus* (Ren-You Gan et al., 2011) and in *Dendrophthoe falcata* (Pattanayak et al., 2011; Patil et al., 2011).

In this study, the ferric reducing antioxidant power (FRAP) in the ethanol extracts of *Dendrophthoe falcata* leaf, tender shoot and bark samples collected from *Artocarpus heterophyllus* host tree, at all concentrations tested, was recorded in the following order: bark > tender shoot > leaf. The FRAP activity was concentration dependent, i.e., it was increased with increasing concentration of extract. The FRAP was higher in the bark extract of *Dendrophthoe falcata* could be due to the presence of more reactive concentration of bioactive constituents and mixture of other compounds in the extract (Table 20) as reported by Pattanayak et al. (2011). Many studies revealed that only polar extracts of plants had showed effective antioxidant activity and some researches further proved that moderate polarity extracts were more potent even if their total phenolic content did not include all the antioxidant (Kahkonen et al., 2001). Vinson et al. (2001) suggested that the synergism among the antioxidant in the mixture made the antioxidant activity not only dependant on the concentration of antioxidant but also on the structure and interaction among the antioxidant.
The DPPH radical is considered to be a model for lipophylic radical. A chain in the lipophilic radical was initiated by the lipid auto-oxidation. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares et al., 1997). The reduction capability of DPPH radical was determined by the decrease in its absorbance at 517 nm which was induced by antioxidants. Hence, DPPH is often used as a substrate to evaluate free radical scavenging activity of antioxidants (Duh et al., 1999). Many reports indicate that the use of DPPH radicals provides an easy, rapid and convenient method to evaluate the antioxidant and radical scavenging. It is a sensitive way to survey the antioxidant activity of a specific compound or plant extracts (Koleva et al., 2002). The DPPH scavenging effect in the ethanol extracts of *Dendrophthoe falcata* leaf, tender shoot and bark samples collected from *Artocarpus heterophyllus* host tree exhibited dose dependent DPPH radical scavenging activity (Table 21) as reported by Motalleb et al. (2005) and Pattanayak et al. (2011). It was noted that all extracts of *Dendrophthoe falcata* samples collected from *Artocarpus heterophyllus* shows a reduction in the DPPH radical scavenging activities than the control, at all concentrations tested (Table 21). The scavenging activity of all samples on the DPPH radicals was found to be strongly dependent on the extract concentration as reported by Motalleb et al. (2005). Among the pant samples tested, the bark extract shows more DPPH-FRS activity than the other sample extracts of *Dendrophthoe falcata*.

Nitric oxide plays an important role in various types of inflammatory processes in the body (Pattanayak et al., 2011). It is a potent diffusible free radical involved in a variety of biological functions, including antimicrobial and anti-tumor activity (Nathan and Gibbs, 1991). Despite the possible beneficial effects of nitric
oxide, its contribution to oxidative damage is also reported. This is due to the fact that nitric oxide can react with superoxide to form the peroxynitrite anion, which is a potential oxidant that can decompose to produce OH- and NO (Pacher et al., 2007). The results of nitric oxide free radical scavenging (NO-FRS) activity showed that nitric oxide radical generated from sodium nitroprusside at physiological pH was found to be initiated by the extracts and it was noted that the percentage of NO-FRS activity was concentration dependent in the leaf, tender shoot and bark samples of Dendrophthoe falcata collected from Artocarpus heterophyllus host tree. Among the plant samples the bark sample showed higher activity than the other sample extracts at all concentrations tested (Table 22).

Superoxide is a reactive oxygen species (ROS), which causes damage to the cells and DNA and leads to various diseases. It was, therefore, proposed to measure the comparative interceptive ability of the antioxidant extracts to scavenge the superoxide radical. Several in vitro methods are available for generation of superoxide radicals (Vani et al., 1997). It is formed by alkaline Dimethyl Sulphoxide (DMSO) which reacts with Nitroblue tetrazolium (NBT) to produce coloured diformazan. It is biologically important as it can form singlet oxygen and hydroxyl radical (Korycka-Dahl and Richardson, 1978). Over production of superoxide anion radical contributes to redox imbalance and associated with harmful physiological consequences (Pervaiz and Clement, 2007). From the results of the present study, it was found that the ethanol extracts of Dendrophthoe falcata leaf, tender shoot and bark samples collected from A. heterophyllus host tree, possessed the superoxide free radical scavenging (SO-FRS) activity and was concentration dependent. Maximum activity
was recorded at high concentration (30µg/ml) of tender shoot extracts as compared to other extracts (Table 23).

The results of the present study are in agreement with the report of Ravishankar et al. (2000) and Mary et al. (2003). However, a large number of phytocompound groups were implicated for antioxidants activity (Devasagayam et al., 2002). They have reported varying levels of antioxidants and free radicals scavenging properties of plant extracts of *Acorus calamus* and *Hemidesmus indicus*. The antioxidant activity is affordable not only by phenolic compound but also has important contributions from other superoxide anion radical scavengers such as essential oils, carotenoids and vitamins (Moure et al., 2001). Some variations in the extent of extract in antioxidant activity were observed for each type of assay used in this study.

The antibacterial and antifungal activity of *Dendrophthoe falcata* as recorded in this study may therefore due to the presence of flavonoids, alkaloids, saponins and tannins in the extract. These phytoconstituents particularly tannins and flavonoids are proven to induce an important antimicrobial activity.

From the results of the present study, it is evident that *Dendrophthoe falcata* leaf, tender shoot and bark samples obtained from *Artocarpus heterophyllus* host tree are rich source of phytocompounds. The presence of various phytocomponents in the samples of *Dendrophthoe falcata* might impart health benefits by combating free radicals in synergistic manner along with other compounds and thus constitute part of the basis for the pharmacological claim. This observation suggests that the phytochemicals, necessary for free radical scavenging activity, are present abundantly
in the extracts of Dendrophthoe falcata samples. All extracts at tested doses, (10 to 30µg/ml for DPPH, NO, and SO radicals) revealed good scavenging activity in a dose dependent manner. The pronounced antioxidant activity of the extracts of Dendrophthoe falcata samples manifested as scavengers of DPPH, nitric oxide, superoxide and ferric reducing power, was possibly due to the various phytocomponents which may act synergistically to exhibit its antioxidant property.

Scientific literature reveals that the carbonyl groups present in the flavonoids and phenolic compounds were responsible for the free radical scavenging activity (Nichols and Budd, 2000). This investigation reveals the presence of pharmacologically active substances like gallic acid, tannins, phenolic compounds, coumarins, etc., responsible for the antioxidant activity.

Bioassay or biological assay is used to estimate the activity or potency of a drug or other substances (plant extracts) by comparing its effects on a test organism with that of a standard preparation. Bioassay is a type of scientific experiment conducted to measure the effects of a substance on living organism and is essential in the development of new drugs and other scientific monitoring. The driving force behind much phytochemical research is the discovery of new biological active compounds for medical or agricultural uses. Biological assays then must be carried out in order to identify promising plant extracts, to guide the separation and isolation, and to evaluate the lead compounds. Identification of natural products from plants that may serve as valuable sources of bioactive agents for medicinal and agricultural uses largely depends on bioactivity directed isolation (Cseke et al., 2006).
Nowadays, herbal plants have been widely used for diseases treatment and immunological enhancement. The increasing trend of herbal application in traditional herbal industry is mainly due to numerous beneficial effects of natural sources compared to single synthetic drug. Natural herbal medicines usually offer less undesirable side effect, more efficiency and less toxic to consumers. However, a very limited scientific data can be accessed regarding the beneficial effect of herbal medicine.

Cytotoxicity studies are widely used by researchers and by pharmaceutical industrie to investigate the toxicity of compounds or extracts on cellular systems. Cytotoxicity assays provide a rapid, sensitive, and validated approach to quantify harmful dose ranges of compounds, and to analyze the biological effects of toxicity on living plant or animal cellular systems.

The principal goal of cytotoxicity study was to determine if extracts from selected medicinal plants and non-medicinal plants were toxic, often, a difference between the therapeutic and a toxic extract or compound is simply the dose level. In this study the toxicity of extracts was determined in both prokaryotic and eukaryotic cells (antimicrobial assay and seed germination assay).

According to reports, the growth of parasitic plants on different kinds of host plants exerts disease curing properties. The evidence of the presence of antimicrobial agents in plants stemmed from the noticeable resistance of such plants to pest attack (Ogundare and Onifade, 2009). Several workers have been carried out in the past to verify the folkloric use of the African mistletoe in the management of microbial infection. Earlier studies by the authors on the crude powder and some of its solvent
fractions have established some significant antibacterial properties, though with negligible antiungal activity (Osadebe and Ukwueze, 2004; Osadebe and Akabogu, 2006; Osadebe et al., 2008; Ukwueze and Osadebe, 2012).

Bark extracts of *Dendrophthoe falcata* showed high antibacterial activity against *E. coli*, *Staphylococcus* sp. and *Pseudomonas* sp. when compared to leaf and tender shoot sample extracts. There is no observable zone of inhibition in leaf extract of *Dendrophthoe falcata* against *E. coli* and *Pseudomonas* sp. The tender shoot extract of *Dendrophthoe falcata* showed less antibacterial activity as compared to the bark extract of *Dendrophthoe falcata*. The activity trend is represented as follows: bark > tender shoot > leaf. The antibacterial activity results equally showed some variations in the level of activity exhibited by the test materials against the organisms tested (Table 24).

The antibacterial activity observed in *Dendrophthoe facata* might have arisen as a result of a number of phytoconstituents present in the plant as reported by Ukwueze et al. (2013). That is, the phytochemical screening results in this study also followed the same trend as above. The higher antibacterial activity might be due to the presence of high concentration of phytoconstituents such as phenols, saponins, tannins and terpenoids in the ethanol bark sample extract of *Dendrophthoe falcata*. The tender shoot sample ethanol extract shows less antibacterial activity than the bark sample extract due to high concentration of phenols and moderate level of alkaloids, coumarins, saponins and tannins and absence of terpenoids. The less antibacterial activity of leaf sample extract than the bark and tender shoot sample extracts might be due to the presence of high concentration of phenol and moderate level of alkaloids,
coumarins and tannins and the absence of saponins and terpenoids. This clearly indicates that no single phytoconstituent could be said to be soly responsible for the antibacterial action of the plant (Ukwueze et al., 2013). Among these constituents screened in this study, however, tannins, flavonoids, terpenoids, and alkaloids appear to have the greatest impact on the activity under review (Osadebe et al., 2004; Osadebe and Ukwueze, 2004; Ukwueze, 2008). These assumptions are in supports with several documented evidence about the medicinal potentials of these plant secondary metabolites. The varied phytochemical constituents (flavonoids, alkaloids, and Saponins) present in the extracts were reported to possess biological activity against microbes (Igbinosa et al., 2009; Narayana et al., 2001).

Many authors have demonstrated the antibacterial activity of these phytochemicals (Hufford et al., 1974; Karou et al., 2006; Takhi et al., 2011). Also some researchers devoted to substances extracted from plants have established that such metabolites like terpenoids, alkaloids, etc., significantly inhibit the growth of bacteria (Escherichia coli, Staphylococcus sps.) and fungi (Ahmed et al., 1993; Habtemariam et al., 1993; Barrey et al., 1997; Hammer et al., 1999; Amaral et al., 1998; Takhi et al., 2011).

Indeed, metabolites like flavonoids and terpenoids are known to be synthesized in response to microbial infection, and thus have been found in vitro to be effective antimicrobial substances against a wide range of microorganisms (Dixon et al., 1983; Himejima et al., 1992; Cowan, 1999a; 1999b). Thus, the observed antibacterial activity in Dendrophthoe falcata may be due to synergy among such constituents like tannins, flavonoids, alkaloids, terpenoids and/or saponins as suggted by Ukwueze et al. (2013).
Leaf extract of *Dendrophthoe falcata* had high antifungal activity against *Pencillum* sp., *Aspergillus niger* and *Aspergillus flavus* as compared to tender shoot and bark extracts (Table 25). Bark extract of *Dendrophthoe falcata* collected from *Artocarpus heterophyllus* host tree showed moderate activity against tested fungi. As observed in this study, Orji *et al.* (2013) reported that the ethanol leaf extract of *Loranthus micranthus* inhibited the growth of *Aspergillus* species and *Penicillium* species which are causative agents of infectious diseases as candidiasis, respiratory micosis, vaginosis, pelvic inflammatory disease, etc., (Cowan, 1999a; 1999b). Various previous reports (Osadebe and Ukwueze; 2004; Osadebe ans Akabogu, 2006; Osadebe *et al.*, 2008; Ukwueze and Osadebe, 2012) indicate the negligible antifungal activity of parasitic plants (*Loranthus micranthus*). The phytochemicals found in the *Dendrophthoe falcata* plant samples confer the antimicrobial properties of the plant. Yusuf *et al.* (2013) reported that the antimicrobial properties was generally found to be more pronounced in the bacteria than in the fungal except for the *Aspergillus* species used. Fungi had been known to possess more complex structure than bacteria and this might confer the resistance on them or provide permeability barrier for the extract to get the fungi. The parasitic plants might have absorbed pharmacological active compounds into their system through their haustorium. The hydroxyl groups of phenol are thought to be responsible for its use as antimicrobial agent (Ogundare and Onifade, 2009), Ademiluyi and Oboh, 2008). However, the optimal harvesting season as well as the host tree of the choice for the parasitic plant determine it as an antimicrobial agent (Lovian, 1980; Osadebe *et al.*, 2008). As suggested by Ukwueze *et al.* (2013), the observed antimicrobial activity in the *Dendrophthoe falcata* plant samples collected from *Artocarpus heterophyllus* host tree might be as a result of
some interactions among the plant constituents rather than that of any one. The antimicrobial activity exhibited by the ethanol extracts of *Dendrophthoe falcata* justifies their use by traditional practitioners in the treatment of sores, boils and open wounds. However, the inability of the leaf extract to inhibit *Escherichia coli* and *Pseudomonas* sp., and the tender shoot extract to inhibit *Pseudomonas* sp. and *Aspergillus flavus* may be that the pathogens possess another mechanism other than acquisition of resistance for detoxifying the active ingredients of the extracts as suggested by Orji *et al.* (2013). The act that some microorganism possesses diverse mechanisms by which they convert substances that inhibit their growth to non-toxic compounds supports this hypothesis (Singleton *et al.*, 1999).

The antibacterial and antifungal activity of *Dendrophthoe falcata* as recorded in this study may therefore due to the presence of flavonoids, alkaloids, Saponins and tannins in the extract. These phytoconstituents particularly tannins and flavonoids are proven to induce an important antimicrobial activity due to their possession of ability to inactivate the microbial adhesions, enzymes, cell envelope transport proteins and so forth (Cowan, 1999a; 1999b).

Allelopathy refers to the beneficial or harmful effects of one plant on another plant, both crop and weed species, from the release of biochemicals, known as allelochemicals, from plant parts by leaching, root exudation, volatilization, residue decomposition, and other processes in both natural and agricultural systems. Allelochemicals are a subset of secondary metabolites not required for metabolism (growth and development) of the allelopathic organism. Allelochemicals with negative allelopathic effects are an important part of plant defense against herbivory (i.e., animals eating plants as their primary food) (Fraenkel 1959; Stamp 2003).
The cytotoxic (allelopathic/inhibitory) nature of the ethanol extracts of *Dendrophthoe falcata* leaf, tender shoot and bark samples obtained from *Artocarpus heterophyllus* host tree was assessed by estimating the percent seed germination and radical growth parameters in the paddy (*Oryza sativa*) and green gram (*Vigna radiate*) seeds treated with the extracts at different concentrations and the observations made on 3rd and 5th day after treatment (Tables 26 to 29). In general, the leaf and bark extracts of *Dendrophthoe falcata* shows less inhibitory effect on the seeds germination of paddy than the green gram, while it was reversed in the tender shoot extract. Effects of allelochemicals on seeds germination appear to be mediated through a disruption of normal cellular metabolism rather than through damage or organelles (Mohamadi and Rajaie, 2009). In conclusion, results of this study showed that the extracts of *Dendrophthoe falcata* have phytotoxic effects on seeds germination and seedling growth of paddy and green gram. The inhibition of seed germination and seeding growth at high concentrations of *Dendrophthoe falcata* may be due to the presence of high concentrations of various phytocompounds screened in this study. Allelopathic potentials of these plants which induces identifying and purification of allelopathic substances.

According to reports, the main reasons behind apparent loss or total absence of any biological activity include the amount at which a particular potential metabolite is produced, synergistic or antagonistic relationship among the molecules when the crude extracts are tested for the biological activities and along with these main reasons, the geographical location of the plants, seasonal variation in the particular area, difference in accumulation of secondary metabolites in different parts of the same plant or time of sample collection are the variable that can significantly alter the yield...
or efficacy of a particular metabolite from a potential plant species (Wagner et al., 1996; Osadebe and Ukwueze, 2004; Ncube et al., 2008). Overall these studies clearly suggest that non-medicinal as well as so-called medicinal plants should be used in general cytotoxicity screening evaluations.