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Screening of Preliminary Phytochemicals and Their Free Radical Scavenging Activities of Ethanolic Extracts of *Toddalia Asiatica* (L.) Lam., *Debregeasia Longifolia* (Burm.F.) Wedd. And *Polygala Arillata* Buch.-Ham Ex Don.

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ABSTRACT
The aim this research paper was to evaluate the phytochemicals and free radical scavenging activities of ethanolic extract of plants like *Toddalia asiatica*, *Polygala arillata*, *Debregeasia longifolia*. The main secondary metabolites such as Alkaloids, Phenols, Flavnoids, Tannins and Saponin have been evaluated among the plants analysed. The antioxidant potential of ethenolic extract of *T. asiatica*, *P. arillata* and *D. longifolia* leaf and stem using tests involving inhibition of lipid peroxidation, hydroxyl radicals, Superoxide anions and 1, 1-diphenyl 2-picrl – hydrazyl free radical (DDPH). The present study revealed that ethanolic extract of Leaf and stem of *T. asiatica*, *P. arillata* and *D. longifolia* are the potential sources of natural antioxidants that could be of greater importance in folkloric medicines.

Key words: Phytochemicals, free radical scavenging activity, *Toddalia asiatica, Polygala arillata, Debregeasia longifolia*

I. Introduction

Medicinal plants are well-known natural sources for the treatment of various diseases since antiquity. About 20,000 plant species used for medicinal purposes are reported by World Health Organisation (WHO, 2002). Despite emphasis being put in research of synthetic drugs, a certain interest in medicinal plants has been reborn, in part due to the fact that a lot of synthetic drugs are potentially toxic and are not free of side effects on the host. This has urged microbiologists all over the world for formulation of new antimicrobial agents and evaluation of the efficacy of natural plant products as a substitute for chemical antimicrobial agents (Balasubramaniam *et al*., 2011).

Toddalia asiatica Lam. (Family–Rutaceae) is a straggler, known as "*Milakarani*" in Tamil, '*Kanchana*' in Sanskrit, and '*Kanj*' in Hindi, used in Indian systems of medicine. There are two varieties; *Toddalia asiatica* var. *gracilis* and var. *floribunda* and they are found in the west and southern parts of India. Plants of var. *gracilis* are generally found in denuded slopes, waste lands and lower hills, whereas that of var. *floribunda* are found only on high altitudes. This plant grows well in clay soils and is widespread in Africa, Asia and Madagascar (Watt and Breyer-Brandwijk, 1962). Milakarani has gained popularity amongst traditional health practitioners for treating numerous ailments. Amongst the documented ethnomedicinal uses, the fruit of this plant is known to have been popularly applied in treating malaria, particularly in East Africa (Orwa *et al*., 2008). The plant has been used in the curing of diseases like paralysis, malarial and intermittent fever, dyspepsia, colic, diarrhea, cough, bronchitis, nausea, wounds, contaminated ulcers, epilepsy, gonorrhoea and general debility (Kirtikar and Basu, 1933; Chopra *et al*., 1956).

These therapeutic values are due to the presence of coumarins, quinoline and benzophenanthridine alkaloids (Bandara *et al*., 1990). Several compounds were isolated from various parts and different extracts of *T. asiatica*. In 1965, Pakrashi and Bhattacharyya reported presence of alkaloids, dihydrochelerythrine and berberin. Deshmukh *et al*., (1976) reported toddalinine, robustine, skimmianine, dictamnine, bergapten, luvangetin and isopimpinelline. Toddasin, Toddanol and Toddanone have been isolated from *T. asiatica* (Sharma *et al*., 1980, 1981). Reis and strobel (1982) have documented coumarins, fatty acid esters, β-sitosterol, alkaloids and some other compounds. A new coumarin, Toddalenone (Ishii *et al*., 1983) and an antiplasmodial coumarin were isolated from *T. asiatica* (Oketch-Rabah *et al*., 2000).

Fruits are eaten for relief from burning sensation in stomach (Ramachandran and Nair, 1981). Leaf and root are used to cure rheumatic swellings and fever pain. The root bark is used medicinally as a tonic and for the treatment of stomach ailments (Watt and Breyer-Brandwijk, 1962), while the leaves are used in patients having lung diseases or rheumatism and

for the treatment of fever, asthma and respiratory diseases (Watt and Breyer-Brandwijk, 1962; Gurib-Fakim *et al*., 1997). The alkaloids of the crude extract have been shown to have anti-inflammatory effects in rats using the carragennan test (Balasubramaniam *et al*., 2011) and to inhibit the auricle swelling caused by xylol and joint swelling caused by agar in rats (Hao, *et al*., 2004). *T. asciatica* are reported to have anti-malarial and anti-leukimatic properties (McCurdy *et al*., 2005; Schlage *et al*., 2000). The central and peripheral antinociceptive effects of *T. asciatica* have been demonstrated using mice (Kariuki *et al*., 2012). Roots as well the leaves are used in parts of East Africa for the management of neuropathic and inflammatory pain (McCurdy *et al*., 2005; Orwa *et al*., 2008). Roots have been shown to be potent in antinociception than leaves (Kariuki *et al*., 2012). Most of the folkloric uses of the genus *T. asiatica* evolve around pain, inflammation and microbial infections. Gakunju *et al*., (1995) have studied the antimalarial activity of the alkaloid Nitidine isolated from *T. asiatica*. The ethanol extract of *T. asiatica* leaf exhibited significant spasmolytic activity (Lakshmi *et al*., 2002). The Kani tribals in southern India use the leaves of *T. asiatica* mixed with coconut oil and three other plants and apply externally to cure skin diseases (Ayyanar and Ignacimuthu, 2005). The root and its bark have been used to treat fever, malaria, cholera, diarrhoea and rheumatism (Duraipandiyan *et al*., 2006). Liu *et al*., (2012) determined larvicidal activity of the essential oil derived from roots of *T. asiatica* (L.) Lam. Stephen *et al*., 2012 assessed the antidiabetic and antioxidant effects of *T. asiatica* leaves ethyl acetate extract in Streptozotocin (STZ) induced diabetic rats.

Polygalaceae, the milkwort family, is the second largest family in the order Fabales, with about 21 genera and some 1,000 species distributed worldwide. A chemical investigation of the genus showed the occurrence of a variety of secondary metabolites, such as xanthones (Pinheiro *et al*., 1998; Yang *et al*., 2000;Cristiano *et al*.,2003;Chaturvedi *et al*., 2005: Linn *et al*., 2005), saponins (Zhang *et al*., 1997; Li and Liang, 1998; Mitaine-Offer *et al*., 2003; Jia *et al*., 2004), oligosaccharides (Jiang and Tu, 2001; Yang *et al*., 2002; Ikeya *et al*., 2004; Li *et al*., 2005), flavonoids (Rao and Raman, 2004; Pizzolatti *et al*., 2008), coumarins (Hamburger *et al*., 1984; 1985; Pizzolatti *et al*., 2000; 2002) and styrylpyrones (Pizzolatti *et al*., 2000; 2004).

Various pharmacological activities have been reported for some species of Polygala, such as anti-inflammatory activity in *Polygala japonica* and *Polygala cyparissias* (El Sayah *et al*., 1999; Kou *et al*., 2006), anxiolytic-like effects in *Polygala sabulosa* (Duarte *et al*., 2007; 2008) trypanocidal activity and antinociceptive effects in *P. sabulosa* and *P. cyparissias* (Pizzolatti *et al*., 2003; 2008; Ribas *et al*., 2008), antibacterial activities in *Polygala myrtifolia* (Lall and Meyer, 1999). In modern pharmacological researches, xanthones had shown various bioactivities, such as anti-inflammatory (Lin *et al*., 1996a), antithrombiotic (Lin *et al*., 1996b), antitumor (Lin *et al*., 1996c; Liu *et al*., 1997) and antimicrobial activities. Xanthone derivatives isolated from *P. cyparissias* have been shown to inhibit neurogenic nociception in rats (De Campos *et al*., 1997). Xanthones with neuro-pharmacological properties have also been reported (Marona *et al*., 1998; Marona 1998). Euxanthone (1,7-dihydroxyxanthone) separated from *polygala caudata* also showed effects on neuronal differentiation (Mak *et al*., 2000). Although the various biological activities of various *Polygala* species have been investigated, the antioxidant properties of *Polygala arillata* have not yet been explored.

Akber *et al*. (2001) reported quercetin, hisperidine, 3b-19alpha-dihydroxy-30-norurs-12-ene,b-sitosterol, stigmasterol, oleanolic acid and lupeol in extracts of *Debregeasia salicifolia*. Ahmed *et al*. (2006) has identified that leaf extracts of *D. salicifolia* has IC_{50} values more than 100 µg/ml of DPPH radical scavenging activity while comparing with Ascorbic acid IC_{50} =1.75

Even though the young leaves and shoots of these plants are popularly used in traditional medicines, there are no reports that provide information on or compare the antioxidant activity of *T. asiatica, D. longifolia and P. arillata* ethanolic extracts. Furthermore, there is no information on the qualitative and quantitative analysis of their active components, which has been reported to be related to antioxidant activity (Hosein and Zinab, 2007). Therefore, this study was undertaken to screen the preliminary phytochemical classes and free radical scavenging activities of leaf and stem ethanolic extracts obtained from of *T. asiatica, D. longifolia and P. arillata* against lipid peroxidation, hydroxyl radicals, superoxide anion radical (O_2^-) and 1, l-diphenyl-2-picryl-hydrazil radical (DPPH.).

II. Materials and Methods

Collection of plant material

The plant materials were collected from the Ghat sections of Palni Hills of Western Ghats. The plant specimens were botanically identified and authenticated by comparing with herbarium specimens and voucher specimens have been deposited in The Rapinat Herbarium (RHT), Tiruchchirappalli. The specimens were deposited under following voucher number viz *Toddalia asiatica* (L.) Lam. var *gracilis* (RHT 56182) RUTACEAE, *Debregeasia longifolia* (Burm.f.) Wedd. (RHT 56184) URTICACEAE and *Polygala*

arillata Buch.-Ham ex Don. (RHT 56183) POLYGALACEAE.

Processing of plant material
The collected plant parts were washed with tap water followed by distilled water. Shade dried plant parts were powdered (100-500 µM) with mechanical blender. 50 g of powder was used for extraction using a Soxhlet apparatus with 300 mL of ethanol. The process was run for 48 h after which the sample was concentrated using rotary evaporator and freeze-dried to powdered form. The dried extracts were weighed and kept in labelled sterile specimen bottles.

Preliminary phytochemical investigations
The major secondary metabolites such as alkaloids, anthraquinones, triterpenes, saponins, phenols and flavonoids were screened according to the methods described by Harborne (1973).

Determination of inhibition of lipid peroxidation
Reaction mixture (0.5 mL) containing plant extract (0.1 mL, 25%) in Tris-HCl buffer (40 mM, pH 7.0), KCl (30 mM), ferrous ion (0.16 mM), and ascorbic acid (0.06 mM) were incubated for 1 h at 37°C in the presence (0.22 to 2.2 mg) and absence of the extracts. The lipid peroxide formed was measured by TBARS formation (Ohkawa *et al*., 1979). Incubation mixtures (0.4 mL) were treated with sodium dodecyl sulfate (SDS; 8.1%, 0.2 mL), thiobarbituric acid (TBA; 0.8%, 1.5 mL), and acetic acid (20%, 1.5 mL, pH 3.5). The total volume was then made up to 4 mL with distilled water and kept in a water bath at 100 °C for 1 h. After cooling, 1 mL of distilled water and 5 mL of a mixture of n-butanol and pyridine (15: 1) were added and vortexed. After centrifugation, the absorbance of the organic layer was measured at 532 nm. The percentage inhibition of lipid peroxidation was determined by comparing the results of the test compound with those of the control not treated with the extract.

Hydroxyl Radical Scavenging Activity
Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and test compound for hydroxyl radicals produced from the Fe3+/ascorbate/ EDTA/H_2O_2 system. The hydroxyl radical attacks deoxyribose, which results in thiobarbituric acid reacting substance (TBARS) formation (Elizabeth and Rao, 1990). The reaction mixture contained deoxyribose (2.8 mM), $FeCl_3$ (0.1 mM), EDTA (0.1 mM), H_2O_2 (1 mM), ascorbic acid (0.1 mM), KH_2PO_4-KOH buffer (20 mM, pH 7.4), and various concentrations of the extract (0.22 to 2.2 mg) in a final volume of 1 mL. The reaction mixture was incubated for 1 h at 37°C. Deoxyribose degradation was measured as TBARS and percentage inhibition was calculated (Ohkawa *et al*., 1979).

Superoxide radical scavenging
This activity was measured by the reduction of NBT according to a previously reported method (Sreejayan and Rao, 1996). The non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS/NADH) system generates superoxide radicals, which reduce nitro blue tetrazolium (NBT) to a purple formazan. The 1 mL reaction mixture contained phosphate buffer (20 µM, pH 7.4), NADH (73 µM), NBT (50 µM), PMS (15 µM) and various concentrations (0–20 µg mL^{-1}) of sample solution. After incubation for 5 min at ambient temperature, the absorbance at 562 nm was measured against an appropriate blank to determine the quantity of formazan generated.

DPPH radical scavenging activity
The antioxidant activity of the extract was evaluated by measuring the ability of the extracts to scavenge the free radical DPPH (Ollanketo *et al*., 2002). For the purpose of comparing the oxidant activity in various extracts, concentration of sample producing 50% reduction of the radical absorbance (IC_{50}) was used as an index. To find this value the extract was diluted in series with ethanol and 2 mL of each added extract was added to 2 mL of 110 µM DPPH solutions. The solution was mixed using a vortex and the mixture was then incubated for 20 h in dark at room temperature, after which the absorbance was measured at the wavelength of 517 nm using ethanol as a reference. The IC50 values were then found from a plot of percent inhibition (PI) versus the concentration. The values of PI was calculated using the following equation: PI(%)=(At/Ar) x 100, where At and Ar are absorbance of test sample and absorbance of the DPPH reference respectively.

Data analysis
Analysis of variance and comparison of means were performed on these antioxidant results to compare the activity between the different methods.

III. Results and Discussion

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (Edeoga *et al*., 2005). The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolics compounds (Hill, 1952). Qualitative preliminary phytochemical analysis their present study confirmed the presence of flavonoids, phenols, carbohydrates, coumarines and steroidal glycoside in the all the tested plant extracts (table-1). All these principles have been known for many years to exhibit biological activity, such as effects on the central nervous system, and antibacterial, antitumour, and anthehelmintic activity,

anti-inflammatory, antitumor activities (Harborne, 1973). However, degree of presence of alkaloids, flavones, tannins and saponins were varying between leaves and stem extracts tested. The observed variation in the extracts found to be containing soluble and non-soluble secondary metabolites or lower than detectable levels by the methodology adopted. The trend was in agreement with the results of Santhi and Jegadeesan (2000). The phytochemical principles such as alkaloid, reducing sugar, glycoside, phyto sterol, phenolics and saponis were found to be absent in petroleum ether, benzene, chloroform extracts and present in ethanol and water extracts of *T. asiatica* leaf and stem. This was further confirmed by Venkata *et al*., (2010), who noticed presence of flavonoids and steroid and absence of alkaloid and terpenoids in the methanolic leaf extract of *T. asiatica*. Anthraquinone was not detected in either stem or leaf of tested plants. The absence of anthraquinone in the *T. asiatica* extracts concurs with the finding of Jeruto *et al*., (2011). They found variation in the chloroform and methanolic root and leaf extracts. Anthraquinone was detected in methanolic root extracts of *T. asiatica* and absent in leaf methanolic and chloroform extracts. The results showed that plant extracts strongly influenced by polarity and solubility of the solvent used.

Quantitative estimations of secondary metabolites of different parts of *T. asiatica, D. longifolia* and *P. arillata* revealed that the extracts of tested parts possessed higher amounts of flavonoids followed by phenols, and tannins as shown in table-2. Mixture of such chemicals shows a spectrum of biological effects and pharmacological properties (Felix, 1982). In general, leaf ethanolic extracts registered higher amount of flavonoids, phenols, and tannins when compared to stem extracts. While higher amount of saponin was registered by stem extracts, the higher amount of total flavonoids was observed in the TAL (*T. asiatica* leaf) 82.1 µg g^{-1} followed by DLL (*D. longifolia* leaf) 47.5 µg g^{-1} and TAS (*T. asiatica* stem 42.6 µg g^{-1}. Similarly total phenol was found to be higher in TAL followed by DLL and TAS. Alkaloids were present in the ethanolic extracts of the tested plants. On this premise it will be advisable to use leaf ethanolic extracts of the tested plants in an attempt to exploit its detoxifying and antihypertensive properties since alkaloids are known to be effective for this purposes (Trease and Evans, 1982). Higher amount of tannins was noticed in leaf ethanolic extracts of TAL<DLL<PAL (*P. arillata* leaf) followed by stem extracts of DLS<TAS<PAS (*P.arillata* stem). Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them (Sodipo *et al*., 1991). The growth of many fungi, yeasts, bacteria and viruses was inhibited by tannins (Chung *et al*., 1998). Presence of tannins suggests the ability of these plants to play a major role for the treatment of many disease (Asquith and Butler, 1986).

Invariably higher amount of saponins was estimated in stem ethanolic extracts when compared to leaf. Among the plant parts tested, DLS extract has higher amount of saponin 29.2 µg g-1 and in PAS 25.9 µg g-1, while higher content of saponin was observed respectively in PAL<DLL<TAL (12.3, 10.2 and 7.0 µg g^{-1}). Saponins are terpene glycosides, useful in pharmaceutical industry in the manufacture of shampoos, insecticides and various drug preparations and in synthesis of steroid hormones (Okwu, 2003). Generally consumption of saponins by human beings may be beneficial in reducing heart disease (by binding of saponins with plasma membrane and cholesterol). According to Shahidul Alam
et al. (2000) the presence of steroidal saponins could develop resistance to viral diseases. Finar (1989) reported that, saponins had expectorant action which is very useful in the management of upper respiratory tract inflammation. So these plants may be used to treat various ailments. Among the plant parts of *T. asiatica, D. longifolia* and *P. arillata* all the crude extract are generally with higher potent of free radicals. The beneficial effects derived from phenolic and flavonoids compounds have been attributed to their antioxidant activity.

Ethanolic extracts of the different plant parts were assessed for their free radical scavenging activities against inhibition of lipid peroxidation, hydroxyl, superoxide and DPPH radicals. The scavenging activity of the ethanolic extracts was tested at concentration ranging between 2.5 to 12.5 µg mL^{-1} against free radicals. IC_{50} value represents the concentration of test extracts where the inhibition of test activity reached 50%. The mean from these antioxidant results were compared with the activity between the different plant parts. The overall results showed that, TA extracts registered higher and highly significant antioxidant activity $P<0.001$ level followed by DL>PA extracts in scavenging free radicals.

Lipid peroxidation has been defined as the biological damage caused by free radical that are formed under oxidative stress. All the tested ethanolic extracts registered highly significant lipid peroxidation activity generated by Fe^{2+}/ascorbate in TBARS. The percentage of inhibition in different concentration of the plant extracts was summarised in table-3. It was observed that higher free radical scavenging activity 85.7 and 82.7% was registered by TAL and DLL at 12.5 µg mL^{-1} followed by PAS extract 69.7%. The lowest concentration needed for 50% of inhibition (IC_{50}) of lipid peroxidation by TAL and DLS extracts was IC_{50} 4.13 and 5.7 µg mL^{-1},

whereas the PAS showed the IC_{50} 8.3 µg mL^{-1}. A concentration dependent inhibition of lipid peroxidation scavenging activity was observed with leaf and stem of the tested plant extracts. When the leaf of TA ethanolic crude extract concentration increased above 10 µg mL^{-1} the lipid peroxidation scavenging activity increased from 73 to 85.7 % respectively at 10 and 12.5 µg mL^{-1}. The antioxidative activity of natural sources is due to the active compounds present in wood, bark, stem, leaf, fruit, root, flower and seed (Pratt and Hudson, 1992).

Degradation of deoxyribose by hydroxyl radicals generated by Fe^{3+}/absorbate/ EDTA/H_2O_2 system was found to be initiated by the extracts of leaf and stem extracts of TA, DL and PA (table-4). A concentration dependent inhibition against hydroxyl radical induced deoxyribose degradation was observed in the deoxyribose assay. The TAL had 85.7% and TAS had 75.3% of hydroxyl radical scavenging activity, similarly DLL 86.3% had higher hydroxyl radical scavenging activity when compared to DAS 82.3%, and PAL the activity was found to be 83.3% and 72.3% in PAS extract at concentration 12.5 µg mL^{-1}. However the highest hydroxyl scavenging activity with lowest IC_{50} value was found in TAS 3.1 µg mL^{-1} followed by DLL 3.4µg mL^{-1} and PAL 3.7µg mL^{-1} respectively (figure-1b). The activity of inhibition showed the order of TS>DL>PL. These results indicates that, the leaf extracts are good source of electron donors, which may accelerate the degradation of deoxyribose by hydroxyl radicals generated by Fe^{3+}/absorbate/EDTA/H_2O_2 system and thus the highest hydroxyl scavenging activity.

Superoxide dismutase is a metallo-protein and is the first enzyme involved in the antioxidant defence by lowering the steady-state level of O_2^-. Peroxidase is an enzyme that catalyzes the reduction of hydroperoxides, including hydrogen peroxides, and protects the cell from peroxidative damage. The ethanolic extracts of TA, DL and PA was found to be having highly significant scavenging activity at $P<0.001$. A concentration dependent inhibition of superoxide radicals generated by riboflavin photoreduction method is shown in table-5. The extracts TAL (82.7%) is followed by PAL and DLL 77.3% having higher SOD activity. The IC_{50} value of the TAL and TAS 6.8 and 8.0 µg mL^{-1} extract DLL and DLS was 8.7 and 10.7µg mL^{-1} respectively whereas the extract of PAS registered IC_{50} 7.6 µg mL^{-1}, and PAL 10.1 µg mL^{-1} (figure-1c).

Free radicals are involved in many human ailments like neurodegenerative diseases, cancer and AIDS. Antioxidants through their scavenging power are useful for the management of those diseases. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts (Koleva *et al*., 2002). The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolonize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for visible deep purple colour. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolourised which can be quantitatively measured from the changes in absorbance.

Among the three plants of leaf and stem extracts tested for the *in vitro* antioxidant activity using the DPPH method (the result was summarised in table 6.) figure 1D shows the amount of each extract needed for 50% inhibition (IC_{50}). The scavenging activity more than 50% was observed with the all the tested crude extracts at varied test substance's concentrations. The TAL and TAS showed strong scavenging activity at 3.19 and 3.27 µg mL^{-1} respectively. However the ethanolic extracts of DLL and DLS showed moderate antioxidant activity with IC_{50} values of 4.16 and 6.17 µg mL^{-1}, than the PAL (6.65 µg mL^{-1}) and PAS (8.86 µg mL^{-1}). The order of scavenging activity found to be TA > DL > PA. This suggested that TA contain compounds such as polyphenolics that can donate electron/hydrogen easily.

The ethanolic leaf extract possessed higher free radical scavenging activity for DPPH, lipid peroxidation, hydroxyl radicals and superoxide. These characteristics of the leaf are probably due to the presence of different phenolics, with diverse antioxidative activities involved in various mechanisms of oxidation inhibition. The presence of glycosides moieties like saponins, phenols and flavonoids which are also known to inhibit tumour growth and serve also to protect against gastrointestinal infections are of pharmacognostic importance and give evidence to the use of the plant in ethnomedicine. Flavonoids on the other hand are water soluble antioxidants and free radical scavengers, which are capable of preventing oxidative cell damage and have strong anticancer activity (Okwu, 2004; Del-Rio *et al*., 1997; Salah *et al*., 1995). The natural antioxidant defence is the repair process, which removes damaged bimolecular entities before they can accumulate and alter cell metabolism. The activity of natural product antioxidants is due to the presence of substituted groups such as carbonyl, phenolic, phytyl side chain, electron withdrawing group, and electron donating group which may be phenolic or non-phenolic (Sailaja *et al*., 2003). In phenolics, the number and position of the phenolic groups decide the antioxidant potential of a compound. And the non-phenolic compounds participate in the antioxidant mechanism through electron transfer and resonance stabilization process (Vinson *et al*., 2001). The study described here suggests that the antioxidative activity exhibited

in ethanolic leaf extracts was not only attributed to the phenolic compounds but also to other non-polar components extracted during the extraction processes. It has also been reported that antioxidant effects were related to various antioxidative components in plants, such as ascorbic acid, carotenoids, tocopherols, chlorophylls, and metal chelating compounds (Takamatsu *et al*., 2003, Chanwitheesuk *et al*., 2005). In addition, antioxidative activities observed in these plants could be the synergistic effect of more than two compounds that may be present in the plant. It has been reported that most natural antioxidative compounds often work synergistically with each other to produce a broad spectrum of antioxidative activities that creates an effective defence system against free radical attack (Lu and Foo, 1995). Further research on the purification and identification of these active compounds would be worthwhile.

IV. Conclusion

The ethanolic extract of *T. asiatica P.arillata* and *D. longifolia* possess a high potent antioxidant activity. The results of preliminary phytochemical screening reveals the presence of alkalaoids, phenols, flavonoids, tunnins saponins etc. from the leaf and stem of plants analysed. The synergistic effect of more than two compounds in the plants tested may develop defense machines against free radicals. Hence this study validates the high therapeutic value of the three selected plants

V. Acknowledgment

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Table-1: Screening of preliminary phytochemical in the ethanolic crude extracts

Phytochemicals	TAL	TAS	DLL	DLS	PAL	PAS
Alkaloids	++	+	++	++	++	+
Terpenoids	++	+	++	+	++	+
Flavonoids	+++	++	++	+	++	+
Flavones	+	+	+	-	+	+
Phenols	+++	++	++	++	++	++
Anthraquinone	-	-	-	-	-	-
Carbohydrate	++	++	++	++	++	++
Coumarins	++	++	++	++	++	++
Tannins	+++	+	+++	++	++	+
Steroidal Glycoside	++	++	++	++	++	++
Saponins	+	++	+	++	+	++

The crude extracts of TAL: *T. asiatica* leaf; TAS: *T. asiatica* stem; DLL: *D. longifolia* leaf; DLS: *D. longifolia* stem; PAL: *P. arillata* leaf; and PAS: *P. arillata* stem.: (–) = absent, (+) = weak, (++) = moderate, (+++) = strong

Table-2: Quantitative estimation of secondary phytochemicals classes in the crude extracts of *T. asiatica, D. longifolia* and *P.arillata* leaf and stem.

Phytochemicals	TAL	TAS	DLL	DLS	PAL	PAS
Alkaloids	15.0	12.1	13.2	11.1	7.0	3.5
Phenols	67.3	40.7	43.4	29.1	39.0	25.1
Flavonoids	82.1	42.0	47.5	19.1	29.2	18.1
Tannins	48.3	15.2	38.0	18.3	27.1	12.0
Saponin	7.0	20.2	10.2	29.2	12.3	25.9

The crude extracts of TAL: *T. asiatica* leaf; TAS: *T. asiatica* stem; DLL: *D. longifolia* leaf; DLS: *D. longifolia* stem; PAL: *P. arillata* leaf; and PAS: *P. arillata* stem. Alkaloids (µg g^{-1}), Phenols (µg g^{-1}), Flavonoids (µg g^{-1}), Tannins (µg g^{-1}) and Saponin (µg g^{-1}) of dry weight.

Table-3: Lipid peroxidation activity of leaf and stem crude extracts of *T. asiatica, D. longifolia* and *P.arillata*.

Conc	TAL	TAS	DLL	DLS	PAL	PAS
2.5	43.0	39.0	26.0	25.0	21.0	25.0
5.0	54.3	43.0	54.3	29.3	29.3	36.3
7.5	64.3	53.3	63.0	38.3	34.0	43.3
10.0	73.0	63.7	78.0	45.0	47.0	57.0
12.5	85.7	70.7	82.7	57.7	56.7	69.7
IC_{50}	4.1	6.3	5.7	10.8	10.9	8.3

Table-4: Hydroxyl Radical scavenging activity of leaf and stem crude extracts of *T. asiatica, D. longifolia* and *P.arillata*.

Conc	TAL	TAS	DLL	DLS	PAL	PAS
2.5	47.3	48.7	41.0	47.7	42.0	32.7
5.0	55.0	53.0	59.3	55.3	58.3	49.3
7.5	60.7	65.3	71.7	63.0	63.7	56.0
10.0	73.0	72.3	81.0	77.7	79.0	67.7
12.5	75.3	85.7	86.3	82.3	83.3	72.3
IC_{50}	3.5	3.1	3.4	3.7	3.7	5.6

Table-5: Superoxide Dismutase activity of leaf and stem crude extracts of *T. asiatica, D. longifolia* and *P.arillata*.

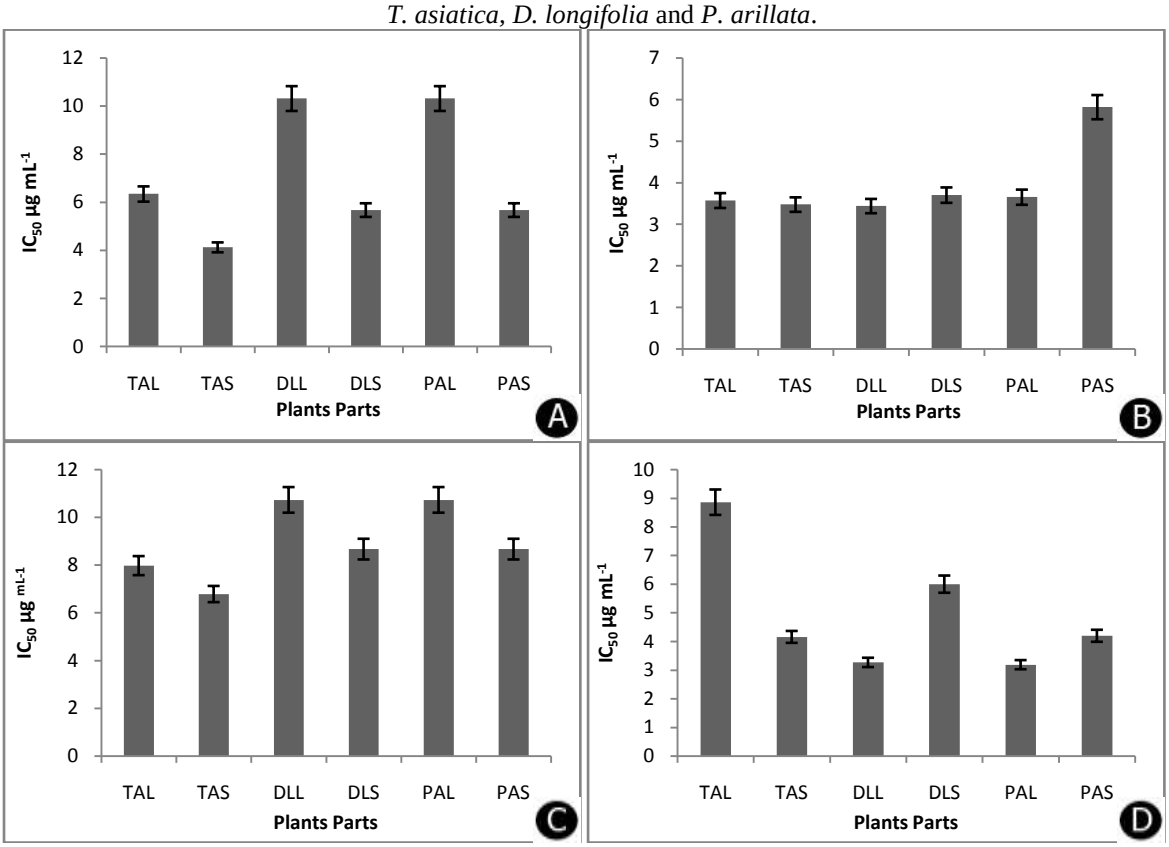
Conc	TAL	TAS	DLL	DLS	PAL	PAS
2.5	31.7	29.7	23.3	25.7	28.3	24.9
5.0	39.0	38.3	27.0	28.7	37.0	29.7
7.5	54.7	44.7	37.3	31.3	49.3	35.3
10.0	60.3	55.7	51.0	44.3	56.0	44.3
12.5	82.7	71.7	77.3	59.3	77.3	63.3
IC_{50}	6.8	8.0	8.7	10.7	7.6	10.1

Table-6: DPPH radical activity of leaf and stem crude extracts of *T. asiatica, D. longifolia* and *P.arillata*.

Conc	TAL	TAS	DLL	DLS	PAL	PAS
2.5	47.2	49.9	48.2	40.0	39.8	44.8
5.0	56.2	55.5	54.4	48.7	45.0	44.2
7.5	58.0	58.7	69.4	53.5	52.2	47.2
10.0	61.9	73.0	60.2	58.7	57.6	50.9
12.5	63.1	77.4	65.4	65.4	66.9	54.8
IC_{50}	3.19	3.27	4.16	6.17	6.65	8.86

Free radicals scavenging activities of leaf and stem crude extracts of *T. asiatica, D. longifolia* and *P.arillata*. TAL: *T. asiatica* leaf; TAS: *T. asiatica* stem; DLL: *D. longifolia* leaf; DLS: *D. longifolia* stem; PAL: *P. arillata* leaf; and PAS: *P. arillata* stem. *Data are expressed as mean percentage and IC_{50} (µg mL^{-1}). Statistical significance at $P < 0.001$.

Figure-1: Free radicals scavenging activities of leaf and stem ethanolic crude extracts of *T. asiatica, D. longifolia* and *P. arillata*.



A. Lipid peroxidation inhibition activity; B. Hydroxyl Radical scavenging activity; C. Superoxide Dismutase activity; and D. DPPH radical scavenging activity of leaf and stem ethanolic crude extracts of *T. asiatica, D. longifolia* and *P.arillata*. TAL: *T. asiatica* leaf; TAS: *T. asiatica* stem; DLL: *D. longifolia* leaf; DLS: *D. longifolia* stem; PAL: *P. arillata* leaf; and PAS: *P. arillata* stem. *Data are expressed as mean percentage ± SE.

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