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Original Research

Total Phenolic Content, Antiradical and Antimicrobial Activity of *Hoya wightii* and *Hoya ovalifolia*

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Abstract

Antiradical and antimicrobial activity of leaf extract of *Hoya wightii* Hook.f. and *Hoya ovalifolia* Wight & Arn. was screened in this study. Antibacterial and antifungal activity of leaf extracts was determined by Agar well diffusion and Poisoned food technique respectively. Antiradical activity was evaluated by DPPH and ABTS radical scavenging assays. The content of total phenolics was estimated by Folin-Ciocalteau reagent method. Extract of *H. ovalifolia* displayed marked antiradical and antimicrobial activity when compared to extract of *H. wightii*. Phenolic content was found to be higher in *H. ovalifolia*. A positive correlation was observed between the phenolic content and antiradical activity of extracts.

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INTRODUCTION

Since the dawn of history, plants have been used for health and food needs by man. Plants are considered to be an important source of carbohydrates, proteins, lipids, minerals and vitamins required for normal growth and development. Besides, plants also produce a number of secondary metabolites such as alkaloids, polyphenolic compounds including flavonoids, terpenoids and tannins which are shown to exhibit health promoting and disease curing properties. These metabolites are distributed in various parts of the plants such as leaves, roots, fruits, seeds, flowers etc. Moreover, these plant metabolites serve as lead compounds for the development of agents with pharmacological activities. Plants have provided valuable drugs like morphine (analgesic), codeine (antitussive), reserpine (antihypertensive), digoxin (cardiotonic), vinblastine and taxol (antineoplastics) and quinine and artemisinin (antimalarials) and most of which are in clinical use. All across the globe, medicinal plants are traditionally used for curing and preventing illnesses as well as promotion of both physical and spiritual well-being among human beings. It is estimated that >80% of world population is dependent on traditional medicine for primary healthcare needs. Plant based formulations are one among the treatment strategies especially in developing and under-developing countries, particularly in remote areas. Plants are integral components of various systems of medicine such as Ayurveda, Sidda and Unani. Many plants have been used to treat diseases of livestock all over the world. Whole plant or various parts of the plants are used traditionally to treat various diseases (Fabricant and Farnsworth, 2001; Ramawat *et al*., 2009; Nwachukwu *et al*., 2010; Parvez and Yadav, 2010; Poornima *et al*., 2012). The species of the genus *Hoya* R. Br. are twining or climbing epiphytic shrubs or herbaceous climbers belonging to the family Apocyanaceae and encompasses numerous species. Most species are growing as epiphytes in the rainforests. The plants are basally woody, and with twining or pendulous stem developing adventitious roots from the internodes. The genus *Hoya* is characterized by persistent inflorescences with flowers having rotate corollas, staminal coronas with revolute margins, pollinia with pellucid margins, and narrow, spindle-shaped seeds without conspicuous wings. The leaves are fleshy and glabrous. Flowers two to numerous in sessile clusters or in peduncled umbel-like cymes (Wanntorp *et al*., 2006a; Wanntorp *et al*., 2006b; Bhat, 2014). In the present study, we evaluated antimicrobial and antiradical activity of *H. wightii* Hook. f. and *H. ovalifolia* Wight & Arn.

MATERIALS AND METHODS

Collection and Identification of Plant Material

H. wightii and *H. ovalifolia* were collected in and around Sagara, Shivamogga district, Karnataka during January 2015. The plants were authenticated by Prof. D. Rudrappa, Dept. of Botany, S.R.N.M.N College of Applied Sciences, Shivamogga.

Extraction

The leaves were separated, washed using clean water and dried under shade. The shade dried leaves were powdered in a blender and extracted by maceration process. 10g of each leaf powder was transferred into clean conical flasks containing 100ml of methanol. The flasks were left for 48 hours with occasional stirrings. The contents of flasks were filtered through muslin cloth followed by Whatman filter paper No. 1. The filtrates were evaporated to dryness at 40^{o}C (Kekuda *et al*., 2015).

Antibacterial Activity of Leaf Extracts

In order to screen antibacterial activity of leaf extracts, Agar well diffusion assay was used. In brief, 24hours old Nutrient broth cultures of test bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*) were swab inoculated on sterile Nutrient agar plates. Wells of 8mm diameter were punched in the inoculated plates and the wells were labeled. The wells were filled with leaf extracts (20mg/ml of dimethyl sulfoxide [DMSO; 25% in sterile distilled water]), reference antibiotic (Chloramphenicol, 1mg/ml of sterile distilled water) and DMSO (25% in sterile distilled water). The plates were incubated in upright position for 24 hours at 37^{o}C. Zones of inhibition formed around wells were measured using a ruler (Kekuda *et al*., 2015).

Antifungal Activity of Leaf Extracts

Poisoned food technique was performed to screen antifungal efficacy of leaf extracts against molds *viz*., *Colletotrichum capsici*, *Alternaria alternata*, *Fusarium oxysporum* f.sp. *zingiberi* and *Aspergillus flavus*. In brief, the test fungi were inoculated aseptically on control (without extract) and poisoned (0.5mg extract/ml) of potato dextrose agar plates by point inoculation method. The plates were incubated at room temperature for 72 hours. Using ruler, colony diameter of test fungi was measured in mutual perpendicular directions after incubation. Antifungal effect, in terms of inhibition of mycelial growth of test fungi, was determined using the formula:

Inhibition of mycelial growth (%) = (A – B / A) x 100,

where 'A' and 'B' refers to colony diameter of test fungi on control and poisoned plates respectively (Kekuda *et al*., 2015).

Antiradical Activity of Leaf Extracts

DPPH Free Radical Scavenging Assay

Different concentrations *viz*., 12.50-200µg/ml of leaf extracts and ascorbic acid (reference standard) were prepared in methanol. 1ml of each concentration of leaf extracts/ascorbic acid was mixed with 3ml of DPPH (1,1-diphenyl-2-picryl-hydrazyl) solution (0.004% in methanol) in clean and dry test tubes. The tubes were incubated in dark for 30 minutes. The absorbance of each tube was measured at 520nm in spectrophotometer. The absorbance of DPPH control (1ml methanol+3ml DPPH solution) was also measured. The scavenging of DPPH radicals (%) by each concentration of extract/reference was calculated using the formula:

Scavenging of DPPH radicals = (C – T / C) x 100, where 'C' and 'T' refers to absorbance of DPPH control and DPPH-extract/reference standard combination. The IC_{50} value was calculated and the value denotes the concentration of extract required to scavenge 50% of DPPH radicals (Kekuda *et al*., 2015).

ABTS Radical Scavenging Assay

The ABTS (2,2-azinobis 3-ethylbenzothiazoline 6-sulfonate) radical was generated by reacting ABTS stock solution (7mM) with potassium persulfate (2.45mM). The reaction mixture was left in the dark for 16 hours at room temperature and the resulting solution was diluted to an absorbance of 0.7 at 730nm using distilled water. Different concentrations *viz*., 12.50-200µg/ml of leaf extracts and ascorbic acid (reference antioxidant) were prepared in methanol. In clean and labeled test tubes, 1ml of different concentrations of leaf extracts (12.5-200µg/ml of methanol) was mixed with 3ml of ABTS radical solution. The tubes were incubated for 30 minutes at room temperature followed by measuring absorbance in a spectrophotometer at 730nm. The absorbance of ABTS control (1ml methanol+3ml ABTS radical solution) was measured. The radical scavenging activity was calculated using the formula:

Scavenging activity (%) = (C - T / C) x 100, where 'C' and 'T' refers to the absorbance of the ABTS radical solution and the absorbance of ABTS radical solution-extract/ascorbic acid combination. The IC_{50} value was calculated and the value denotes the concentration of extract required to scavenge 50% of ABTS free radicals (Kekuda *et al*., 2015).

Total Phenolic Content of Extract

Total phenolic content was estimated by Folin-Ciocalteau reagent (FCR) method (Kekuda *et al*., 2015). Here, a dilute concentration of each leaf extract (0.5 ml) was mixed in separate tubes with 0.5 ml of FC reagent (1:1) and 2 ml of sodium carbonate (2%) and the tubes were incubated for half an hour at room temperature. The absorbance was measured at 765nm in a spectrophotometer. A standard curve was plotted using different concentrations (0-1000 µg/ml) of Gallic acid (standard). From the graph, the total phenolic content of leaf extracts was estimated and expressed as mg Gallic Acid Equivalents (GAE).

RESULT AND DISCUSSION

Antibacterial Activity of Leaf Extracts

The discovery of antibiotics is considered as one of the most significant discoveries in medicine. However, the indiscriminate use of these antibiotics resulted in emergence of resistant pathogens. Antibiotic resistance appears to be a global problem. Besides, the spread of these resistant bacteria in hospital and community settings is a burden to health services. Moreover, these drugs have drawbacks such as high cost and adverse side effects. Due to these, search for new antimicrobials from other sources is of much interest. Plants have been traditionally used to treat various diseases since time immemorial. Plants produce a wide array of secondary metabolites exhibiting therapeutic potentials. These metabolites can either inhibit the growth of bacteria or kill them, with no or least toxicity to host (Adwan *et al*., 2010; Zwetlana *et al*., 2014; Jr. Valle *et al*., 2015; Kekuda *et al*., 2015). Table 1 shows the antibacterial effect of leaf extract of *H. wightii* and *H. ovalifolia*. Both extracts exhibited inhibitory efficacy against test bacteria. Extract of *H. ovalifolia* displayed higher inhibitory activity when compared to *H. wightii*. Extract of *H. wightii* caused least inhibition of *E. coli* while other bacteria were inhibited to similar extent. Similarly, *E. coli* and *B. subtilis* were inhibited to least extent by extract of *H. ovulifolia*. Overall, *P. aeruginosa* was susceptible to higher extent to leaf

extracts. Standard antibiotic caused marked inhibition of test bacteria when compared to leaf extracts. There was no inhibition of test bacteria in case of DMSO.

Table 1: Antibacterial activity of extracts of *H. wightii* and *H. ovalifolia*

Test Bacteria	Zone of inhibition in cm			
	H. wightii	***H. ovalifolia***	**Antibiotic**	**DMSO**
E. coli	0.8	1.1	2.2	0.0
P. aeruginosa	1.0	1.3	2.6	0.0
S. aureus	1.0	1.2	3.2	0.0
B. subtilis	1.0	1.1	3.0	0.0

Antifungal Activity of Leaf Extracts

Fungal infections of plants are among the most devastating microbial infections leading to low productivity and crop loss (in many cases >50%). The pre- and post-harvest infections result in major economic losses to farmers. Chemical fungicides are routinely used against fungal pathogens. However, these chemicals are costly and their use results in emergence of resistant strains, residual effect in environment and toxic effect against non-target organisms including humans. The need for developing antifungal agents from natural sources having least or no side effects is of much worth. It has been shown that plants and their metabolites exhibit inhibitory effect against a range of phytopathogenic fungi (Park *et al*., 2008; Dellavalle *et al*., 2011; Panea *et al*., 2013; Kekuda *et al*., 2014; Vivek *et al*., 2014; Kekuda *et al*., 2015; Shweta *et al*., 2015). In this method, we evaluated antifungal efficacy of leaf extracts by poisoned food method and the result is depicted in Table 2. Poisoning of the medium resulted in reduction in mycelial growth of test fungi. Both extracts inhibited fungi to a varied extent. Among extracts, *H. ovalifolia* displayed higher antifungal effect when compared to *H. wightii*. Extract of *H. wightii* caused highest and least inhibition of *C. capsici* and *A. flavus* respectively whereas *A. flavus* and *C. capsici* were inhibited higher and least extent by extract of *H. ovalifolia*. None of the fungus was inhibited to 25% or higher by both the extracts.

Table 2: Antifungal activity of extracts of *H. wightii* and *H. ovalifolia*

Test fungi	Colony diameter in cm (% inhibition)		
	Control	***H. wightii***	***H. ovalifolia***
C. capsici	3.2	2.8 (12.50)	2.7 (15.62)
A. alternata	2.8	2.6 (7.14)	2.3 (17.85)
F. oxysporum	4.8	4.3 (10.41)	4.0 (16.66)
A. flavus	3.4	3.2 (5.88)	2.6 (23.52)

DPPH Radical Scavenging Activity of Leaf Extracts

Several in vitro assays are available for evaluating free radical scavenging of samples. Among these, DPPH assay is widely used as it is simple, rapid and the results are reproducible. DPPH is a stable, organic, nitrogen centred, purple colored free radical showing maximum absorption at 515-520nm in alcoholic solution. In the presence of an antioxidant, the radical loses the absorption as it is reduced to DPPHH (purple color changes to yellow). The assay is routinely used to evaluate radical scavenging activity of plant extracts (Chung *et al*., 2006; Wan *et al*., 2011; Rakesh *et al*., 2013; Shalaby and Shanab, 2013). Figure 1 shows the scavenging of DPPH radicals by leaf extracts. Both extracts scavenged radicals in a dose dependent manner. Extract of *H. ovalifolia* scavenged DPPH radicals more efficiently (IC_{50} value 120.25µg/ml) when compared to *H. wightii* (IC_{50} value 340.89µg/ml). At 12.50µg/ml concentration, extract of *H. wightii* did not show scavenging of the radicals. At concentration 200µg/ml, a scavenging of 28.73 and 79.31% was observed in case of *H. wightii* and *H. ovalifolia* respectively. Reference standard i.e., ascorbic acid displayed marked scavenging effect (IC_{50} value 3.27µg/ml) when compared to leaf extracts. It is evident from this *in vitro* assay that the leaf extracts possess the hydrogen donating property which possibly makes the extract to scavenge free radicals and thereby acting possibly as primary antioxidants (Chung *et al*., 2006).

ABTS Radical Scavenging Activity of Leaf Extracts

The scavenging efficacy of leaf extracts was also tested using ABTS radical scavenging assay. This *in vitro* assay is another widely used radical scavenging assay. Unlike DPPH assay, this assay involves the generation of ABTS radicals (by reacting the ABTS salt with a strong oxidizing agent such as potassium permanganate or potassium persulfate). An electron donating compound reduces the blue-green ABTS radical to colorless neutral form. The assay of ABTS scavenging is simple, rapid and is routinely used to evaluate radical scavenging potential of several kinds of compounds including plant extracts (Wan *et al*., 2011; Rakesh *et al*., 2013; Shalaby and Shanab, 2013). Figure 2 shows the scavenging of ABTS radicals by leaf extracts. The leaf extracts scavenged radicals in a concentration dependent manner. Here also, extract of *H. ovalifolia* scavenged radicals more efficiently (IC_{50} value 49.94µg/ml) when compared to *H. wightii* (IC_{50} value 167.26µg/ml). At 12.50µg/ml concentration, extract of *H. wightii* showed least scavenging of the radicals (5.88%). At concentration 200µg/ml, a scavenging of 52.94 and 94.11% was observed in case of *H. wightii* and *H. ovalifolia* respectively. Reference antioxidant i.e., ascorbic acid showed high scavenging effect (IC_{50} value 2.17µg/ml) when compared to leaf extracts. Although leaf extracts have shown low scavenging of ABTS radicals than that of ascorbic acid, it is confirmed that the extracts possess electron donating property and hence the extracts could serve as free radical scavengers.

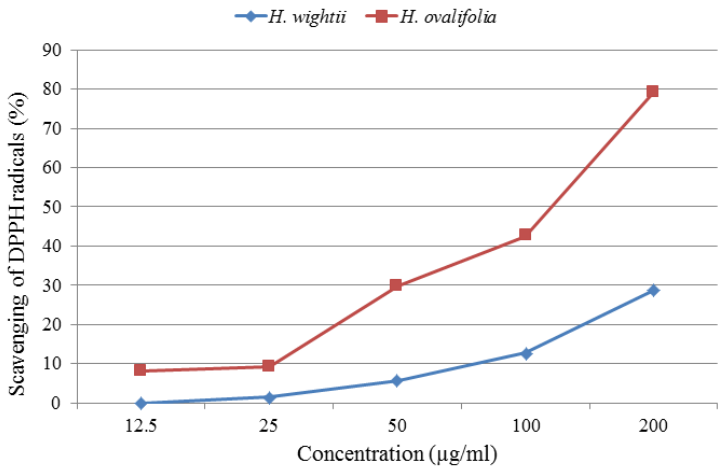

Figure 1: Scavenging of DPPH radicals by extracts of *H. wightii* and *H. ovalifolia*

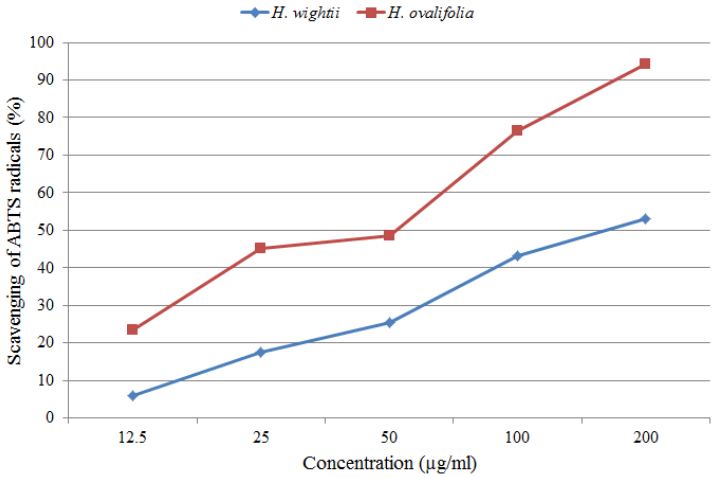

Figure 2: Scavenging of ABTS radicals by extracts of *H. wightii* and *H. ovalifolia*

Total Phenolics in Leaf Extracts

Plants produce various secondary metabolites in response to several stimuli. Among these, polyphenolic compounds including flavonoids are shown to display a wide array of bioactivities including antioxidant activity. These compounds are distributed in various parts of the plants such as leaves, roots, flowers, seeds etc. Phenolic compounds play an important role in stabilizing lipid oxidation. Due to their antioxidant function, phenolic compounds may exhibit inhibitory effects on mutagenesis and carcinogenesis in humans. The antioxidant properties of phenolic compounds can be ascribed to various mechanisms such as reactive oxygen species scavenging, inhibition of the generation of free radicals and chain-breaking activity and metal chelation. FCR method is the oldest and widely used *in vitro* assay for estimation of total phenolic content. Under basic conditions (adjusted using sodium carbonate), the phenolic compounds interact with FCR producing a blue colored complex having maximum absorption near 750nm. This method is simple, reliable, convenient and the results obtained are reproducible (Chung *et al*., 2006; Chang *et al*., 2007; Gulcin *et al*., 2011; Rakesh *et al*., 2013; Kekuda *et al*., 2015). The content of total phenolics in the leaf extracts was estimated by FCR method and the result was expressed in terms of mg GAE/g of extract. The extract of *H. ovalifolia* was found to contain high total phenolics (36.26 mg GAE/g) when compared to extract of *H. wightii* (22.13 mg GAE/g). A direct correlation is observed between the radical scavenging activity and the phenolic content i.e., extract of *H. ovalifolia* containing high phenolics displayed marked scavenging effect when compared to extract of *H. wightii* which was found to possess less phenolic content. Similar results were obtained in earlier studies of Tilak *et al*. (2004), Coruh *et al*. (2007), Kekuda *et al*. (2012), Rakesh *et al*. (2013) and Pavithra *et al*. (2013) where extracts containing high phenolics exhibited high radical scavenging activity.

CONCLUSIONS

Overall, extract of *H. ovalifolia* displayed stronger bioactivities when compared to extract of *H. wightii*. The higher bioactivity of *H. ovalifolia* could be ascribed to the presence of high phenolic content. Although the bioactivity shown by extracts *in vitro* was low, it is evident that the extracts possess bioactive principles which are to be isolated, characterized and subjected for bioactivity determinations.

Conflict of Interest

Conflict of interest none declared.

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REFERENCES

Adwan, G., Abu-Shanab, B., Adwan, K. (2010). Antibacterial activities of some plant extracts alone and in combination with different antimicrobials against multidrug–resistant *Pseudomonas aeruginosa* strains. *Asian Pacific Journal of Tropical Medicine* 3(4): 266-269.

Bhat, G.K. (2014). Flora of South Kanara (Dakshina Kannada and Udupi districts of Karnataka). Aakrithi Prints, Mangalore, India, 685-686.

Chang, H., Ho, Y., Sheu, M., Lin, Y., Tseng, M., Wu, S., Huang, G., Chang, Y. (2007). Antioxidant and free radical scavenging activities of *Phellinus merrillii* extracts. *Botanical Studies* 48: 407-417.

Chung, Y., Chien, C., Teng, K., Chou, S. (2006). Antioxidative and mutagenic properties of *Zanthoxylum ailanthoides* Sieb & zucc. *Food Chemistry* 97: 418-425.

Coruh, N., Celep, A.G.S., Ozgokce, F., Iscan, M. (2007). Antioxidant capacities of *Gundelia tournefortii* L. extracts and inhibition on glutathione-S-transferase activity. *Food Chemistry* 100: 1249-1253.

Dellavalle, P.D., Cabrera, A., Alem, D., Larranaga, P., Ferreira, F., Rizza, M.D. (2011). Antifungal activity of medicinal plant extracts against phytopathogenic fungus *Alternaria* spp. *Chilean Journal of Agricultural Research* 71(2): 231-239.

Fabricant, D.S., Farnsworth, N.R. (2001). The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspectives* 109(S1): 69-75.

Gulcin, I., Topal, F., Sarikaya, S.B.O., Bursal, E., Bilsel, G., Goren, A.C. (2011). Polyphenol contents and antioxidant properties of Medlar (*Mespilus germanica* L.). *Records of Natural Products* 5(3): 158-175.

Jr. Valle, D.L., Andrade, J.I., Puzon, J.J.M., Cabrera, E.C., Rivera, W.L. (2015). Antibacterial activities of ethanol extracts of Philippine medicinal plants against multidrug-resistant bacteria. *Asian Pacific Journal of Tropical Biomedicine* 5(7): 532-540.

Kekuda, P.T.R., Akarsh, S., Darshini, S.M., Prafulla, D., Raghavendra, H.L. (2015). Antiradical and antimicrobial activity of *Atylosia lineata* Wt. and Arn. *Science, Technology and Arts Research Journal* 4(3): 180-183.

Kekuda, P.T.R., Rakesh, K.N., Dileep, N., Junaid, S., Pavithra, G.M., Gunaga, S.S., Megha, V.H., Raghavendra, H.L. (2012). Antimicrobial and antioxidant activity of *Anaphalis lawii* (Hook.f.) Gamble. *Science Technology and Arts Research Journal* 1(3): 8-16.

Kekuda, P.T.R., Vivek, M.N., Manasa, M., Kambar, Y., Nawaz, N.A.S., Raghavendra, H.L. (2014). Antifungal effect of cow urine extracts of selected plants against *Colletotrichum capsici* isolated from anthracnose of chilli. International *Journal of Agriculture and Crop Sciences* 7(3): 142-146.

Nwachukwu, C. U., Umeh, C.N., Kalu, I.G., Slyvester, O., Magnus, N. (2010). Identification and traditional uses of some common medicinal plants in Ezinihitte Mbaise L.G.A., of Imo state, Nigeria. *Report and Opinion* 2(6): 1-8.

Panea, C., Piccolo, A., Spaccini, R., Celanoc, G., Villeccoa, D., Zaccardellia, M. (2013). Agricultural waste-based composts exhibiting suppressivity to diseases caused by the phytopathogenic soil-borne fungi *Rhizoctonia solani* and *Sclerotinia minor*. *Applied Soil Ecology* 65: 43-51.

Park, I., Kim, J., Lee, Y., Shin, S. (2008). In vivo fungicidal activity of medicinal plant extracts against six phytopathogenic fungi. *International Journal of Pest Management* 54(1): 63-68.

Parvez, N., Yadav, S. (2010). Ethnopharmacology of single herbal preparations of medicinal plants in Asendabo district, Jimma, Ethiopia. *Indian Journal of Traditional Knowledge* 9(4): 724-729.

Pavithra, G.M., Siddiqua, S., Naik, A.S., Kekuda, P.T.R., Vinayaka, K.S. (2013). Antioxidant and antimicrobial activity of flowers of *Wendlandia thyrsoidea*, *Olea dioica*, *Lagerstroemia speciosa* and *Bombax malabaricum*. *Journal of Applied Pharmaceutical Science* 3(6): 114-120.

Poornima, G., Manasa, M., Rudrappa, D., Kekuda, P.T.R. (2012). Medicinal plants used by herbal healers in Narasipura and Manchale villages of Sagara Taluk, Karnataka, India. *Science, Technology and Arts Research Journal* 1(2): 12-17.

Rakesh, K.N., Junaid, S., Dileep, N., Vinayaka, K.S., Kekuda, P.T.R., Raghavendra, H.L. (2013). Antibacterial and antioxidant activity of *Fahrenheitia zeylanica* (Thw.) Airy. *Science, Technology and Arts Research Journal* 2(4): 27-33.

Ramawat, K.G., Dass, S., Mathur, M. (2009). The chemical diversity of bioactive molecules and therapeutic potential of medicinal plants. In: Herbal Drugs: Ethnomedicine to Modern Medicine. Ramawat, K.G. (editor), Springer-Verlag Berlin Heidelberg, pp 7-32.

Shalaby, E.A., Shanab, S.M.M. (2013). Comparison of DPPH and ABTS assays for determining antioxidant potential of water and methanol extracts of *Spirulina platensis*. *Indian Journal of Geo-Marine Sciences* 42(5): 556-564.

Shweta, S.D., Sudeshna, C.S., Rashmi, K., Vrushala, P.S., Kekuda, P.T.R. (2015). Antifungal efficacy of some epiphytic orchids of Karnataka, India. *Scholars Journal of Agriculture and Veterinary Sciences* 2(3B): 266-269.

Tilak, J.C., Adhikari, S., Devasagayam, T.P.A. (2004). Antioxidant properties of *Plumbago zeylanica*, and Indian medicinal plant and its active ingredient, plumbagin. *Redox Report* 9(4): 220-227.

Vivek, M.N., Manasa, M., Kambar, Y., Kekuda, P.T.R., Raghavendra, H.L. (2014). Antifungal efficacy of three bioactive *Parmotrema* species from Western Ghats of Karnataka, India. *International Journal of Agriculture and Crop Sciences* 7(12): 968-973.

Wan, C., Yu, Y., Zhou, S., Liu, W., Tian, S., Cao, S. (2011). Antioxidant activity and free radical-scavenging capacity of *Gynura divaricata* leaf extracts at different temperatures. *Pharmacognosy Magazine* 7(25): 40-45.

Wanntorp, L., Kocyan, A., Renner, S.S. (2006b). Wax plants disentangled: A phylogeny of *Hoya* (Marsdenieae, Apocynaceae) inferred from nuclear and chloroplast DNA sequences. *Molecular Phylogenetics and Evolution* 39: 722-733.

Wanntorp, L., Kocyan, A., van Donkelaar, R., Renner, S.S. (2006a). Towards a monophyletic Hoya (Marsdenieae, Apocyanaceae): Inferences from the chloroplast *trnL* region and the *rbcL-atpB* spacer. *Systematic Botany* 31(1): 586-596.

Zwetlana, A., Nandini, M., Dorcas, K. (2014). Antimicrobial activity of medicinal plant extracts on gram negative bacteria. *Journal of Medicinal Plants Studies* 2(5): 51-54.