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Antioxidant, antimicrobial activities and phytochemical analysis of leaves extracts of *Dioscorea wallichii* Hook. f.

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ABSTRACT

The present study was carried out to evaluate the phytochemical, antioxidant activity and antimicrobial activity of leaf of *Dioscorea wallichii*. The antimicrobial effect of the studied plant was evaluated on the following bacterial species, *Micrococcus mucilaginosus, Escherichia coli, Klebsiella terrigena*, *Pseudomonas aeruginosa, Bacillus cereus*, *Staphyllococcus aureus,* and *Candida albicans.* The solvents used for the extraction were acetone, ethyl acetate and methanol. The *in vitro* antimicrobial activity was performed by well diffusion method. The antimicrobial activities of *D. wallichii* in successive different solvent were tested against Gram positive and Gram negative bacteria and fungus. The extracts showed the presence of flavonoids, coumarins, phenols, tannins, saponins, terpenoids, quinones and catechins. The acetone and methanol extracts of *D. wallichii* showed the broad spectrum of antimicrobial activity when compared with positive control. The DPPH assay was also conducted for methanolic leaf extract exhibited highest activity which is compare to the activity of the ascorbic acid standard. In conclusion, all organic crude extracts from leaves could be used as potential sources of new antioxidant and antimicrobial agents.

INTRODUCTION

Medicinal plants are the important source of natural antioxidants. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species (ROS) such as superoxide, hydroxyl radicals, singlet oxygen, peroxy radicals etc. These active oxygen species and free radicals can attack molecules in biological membranes and tissues and thus inducing oxidative stress that further has been associated with cancer, ageing, inflammation, neurodegenerative diseases, hypertension, artherosclerosis (Stocker and Keaney, 2004; Devasagayam *et al*., 2004; Kaur *et al*., 2007; Grassi *et al*., 2009). Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. The main characteristic of an antioxidant is its ability to trap free radicals (Walton and Brown, 1999).

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The major antioxidants of vegetables are vitamins C and E, carotenoids and phenolic compounds, especially flavonoids. These compounds present in vegetables contribute free radicals scavenge and inhibit the chain initiation or break the chain propagations give both to the first and second defense lines against oxidative stress. As a result, they protect cells against oxidative damage, and may therefore prevent chronic diseases, such as cancer, cardiovascular disease, and diabetes. Vitamin E and carotenoids also contribute to the first defense line against oxidative stress, because they quench singlet oxygen (Krinsky, 2001; Shi *et al.*, 2001).

Free radicals cause several disorders, including diabetes, and the agents that scavenge free radicals may have great potential in ameliorating these diseases (Wilson *et al.*, 1988). The most frequently encountered free radicals are the hydroxyl radical ($HO^{\bullet}$), the superoxide radical (O_2•-), the nitric oxide radical (NO•) and the lipid peroxyl radical (LOO•) while non-free radical species principally being H_2O_2 and singled oxygen ($^1O_2$) (Yildirim *et al*., 2000). *Dioscorea wallichii* Hook.f. belongs to the family Dioscoreaceae and used as a major food supplements. It is found in India, Bangladesh, Burma, Thailand and China.

It climbs on large shrubs or trees in mixed deciduous and evergreen montane forests. Tubers of *D. wallichii* are consumed on a local scale as a source of carbohydrates. They are eaten by ethnic groups such as the Sakai tribe, who live in the Banthad Range in Peninsular Thailand. These are also eaten by tribes in Orissa, India, during the winter months (Maneenoon *et al*., 2008). Rout and Panda, (2010) reported that, various tribal communities in Mayurbhanji district of Odisha using root extract of this plant for curing stomach pain. Till date there are no reports published about the phytochemical analysis and antimicrobial activity of the leaves extract of *D. wallichii*. Therefore this study was carried out to evaluate the phytochemical content, antioxidant and antimicrobial activity of different solvents extracts of the leaves of *D. wallichii*.

MATERIAL AND METHODS

Plant material

Leaves samples of *D. wallichii* plants were collected from Courtallum hills, Tirunelveli District, Tamil Nadu, India during the month of October 2015. The plant was identified and the herbarium specimen was prepared and deposited at Botany research laboratory, V.H.N.S.N. College (Autonomous) for future references.

Preparation of leaves extracts

The fresh leaves were air dried under fan and the leaves extracts were prepared by sequential extraction method using three organic solvents on the basis of the polarity of solvents (Acetone, Ethyl acetate and Methanol). 30g of the dried leaves sample was taken in a conical flask and 200 ml of Acetone was added. The conical flask was kept on mechanical shaker for 24 hours, after that the extract was filtered through Whatman filter paper 1 and the pellet was allowed to drying and this pellet was used for the next solvent extraction (Ethyl acetate and Methanol). The dried extract was recovered and stored in refrigerator for further analysis.

Phytochemical Screening

Preliminary Phytochemical Screening

The leaves extracts of *D. wallichii* were qualitatively analysed to find out the presence of phenols, flavonoids, quinones, alkaloids, saponins, tannins, steroids, terpenoids, coumarins and catechins following standard methods (Kokate, 2000; Harborne, 1999; Edeoga *et al*., 2005; Harbone, 1973; Yadav *et al*., 2014 and Gopinath *et al*., 2012).

Quantitative phytochemical analysis

Estimation of total phenol content

The amount of total phenol was determined using the Folin-Ciocalteu reagent method of Lister and Wilson, 2001. A standard curve was prepared by using gallic acid. Different concentrations of gallic acid were prepared in 80% methanol, and their absorbance was recorded at 760 nm. 100µl of sample was dissolved in 500µl of Folin-Ciocalteu reagent (1/10 dilution) and 1ml of distilled water. The contents were mixed and incubated at room temperature for 1 min. After 1 min, 1.5 ml of 20% sodium carbonate solution was added. The final mixture was shaken well and incubated for 2h in the dark at room temperature. The absorbance of all samples was measured at 760 nm using a UV-Vis spectrophotometer. The results were expressed in mg gallic acid equivalents (GAE) per milligram of dry weight of the plant.

Estimation of total flavonoid content

The flavonoid content in the extract was determined spectrophotometrically by the method of Quettier-Deleu *et al*., 2000. This method was based on the formation of a complex, flavonoid-aluminium, with the absorbance maximum at 430 nm. Rutin was used as standard to make the calibration curve. 1ml of diluted sample was separately mixed with 1ml of 2% aluminium chloride methnolic solution. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm in a UV-Vis spectrophotometer. The flavonoid content was expressed in mg per mg of rutin equivalent (RE).

Free Radical Scavenging Ability (DPPH)

The scavenging ability of methanol extract on 1, 1-diphenyl-2-picrylhydrazyl free radicals was estimated according to the method of Shimada *et al*., (1992). This method depends on the reduction of purple DPPH to yellow colored diphenyl picryl hydrazine. 2 ml of various concentrations (10-100 µg/ml) of test sample was mixed with 0.5 ml of 0.005M DPPH in methanol. An equal amount of methanol and DPPH served as a control. The mixture was shaken vigorously and then steadily kept for 30 min at room temperature in dark. The absorbance of the resulting solution was measured at 517 nm against a blank using UV-Vis spectrophotometer. The experiment was performed in triplicates. The DPPH radical scavenging activity was calculated by the following equation;

$$\% \text{ DPPH radical scavenging activity} = (A_o - A_1)/A_o \times 100\%$$

Where A_o is the absorbance of the control reaction and A_1 is the absorbance of the sample of the tested extracts. Percentage of free radical activity was plotted against the corresponding antioxidant substance concentration to obtain the IC_{50} value, which is defined as the amount of antioxidant substance required to scavenge the 50% of free radicals present in the assay solution. IC_{50} values are inversely proportional to the antioxidant potential.

Antimicrobial activity

Extracts were screened against six bacterial species: *Bacillus cereus* (GRIBI01), *Pseudomonas aeruginosa* (GRIBI04), *Staphylococcus aureus* (GRIBI05), *Escherichia coli* (GRIBI06) *Klebsiella terrigena* (GRIBI08), *Micrococcus mucilaginosus (GRIBI09) and* a fungus *Candida albicans* (GRIBI03). All strains were obtained from the Department of Biology Gandhigram Rural Institute, Dindigul. The test organisms were maintained on nutrient

agar slant and kept in a refrigerator at 4°C. 100ml aliquots of nutrient broth were inoculated with the culture of test micro-organisms using a loop and then incubated at 37°C for 24 hrs. Antimicrobial activities of methanol, ethyl acetate and acetone fractions of *D. wallichii* were carried out using the agar well diffusion method. Mueller-Hinton agar medium (MHA) was used for antimicrobial susceptibility tests. The MHA medium was prepared by pouring 20 ml of molten media into sterile Petri plates. The plates were allowed to solidify and 100µl of an overnight broth culture of test micro-organisms was swabbed uniformly on the medium and allowed to dry for 5 min. For agar well diffusion method, four equidistant wells (6 mm in diameter) were cut from the agar with the help of a cork-borer. 40 µl of leaves extracts (methanol, ethyl acetate and acetone extracts) containing 4 mg concentration was loaded on 6 mm well. The standard antibiotic disc Gentamicin (10mcg/disc) was placed on the surface of the plates. The plates were kept for incubation for 24 hrs at 37°C. The zone of inhibition was measured around the well containing samples and standard. The experiments were performed in triplicates.

Statistical analysis

All the data was reported as mean ± standard deviation of three replicates. The IC_{50} values were calculated using the ED_{50} plus v 1.0 programme. Statistical analysis was performed using Microsoft Excel.

RESULTS

Preliminary phytochemical analysis

The results of phytochemical analysis of leaves *D. wallichii* showed the presence of alkaloids, flavonoids, coumarins, phenols, tannins, saponins, terpenoids, quinones and catechins. The phytoconstituents steroids, quinones and catechins were not reported in any of the plant extract. All the extracts were reported to show positive result in color reaction only for alkaloids, flavonoids and saponons. Acetone and Ethyl acetate extract no responsible to the quinones, catechins and coumarins colouring tests. The phytochemical constituents of the plants investigated are summarized in Table 1.

Table 1: Phytochemical characterization of extracts from the leaves of *D. wallichii.*

Plant solvent extraction	Alkaloids	Flavonoids	Phenols	Saponins	Tannins	Terpenoids	Quinones	Catechins	Coumarins	Steroids
Acetone	+	+	-	+	-	-	-	-	-	-
Ethyl acetate	+	+	+	+	+	-	-	-	-	-
Methanol	+	+	+	+	+	+	-	-	+	-

+ = indicates presence of phytochemicals, - = indicates absence of phytochemicals.

Total phenol and flavonoid content

Table 2. shows total content of phenolic compounds in the studied plant extracts expressed in equivalent of gallic acid mg/mg of plant extract. The highest amount of phenolic compounds (0.486±0.098 mg GAE/mg) was found in the ethyl acetate extract and compared with other samples, whereas the acetone extract contained the lowest level of TPC (0.190±0.02 mg GAE/mg). The level of flavonoids, expressed in Rutin equivalents (REq) in mg/mg of plant extract, varied from 0.212±0.024 mg REq /mg, 0.201±0.023 mg REq /mg and 0.144±0.006 mg REq/mg in methanol, ethyl acetate and acetone extracts. The highest amount was found for the methanol extract.

Table 2: Quantitative phytochemical analysis of three solvent extracts of *D. wallichii.*

Phytochemicals constituents	Methanol extract	Acetone extract	Ethyl acetate extract
Phenols (mg GAE/mg)	0.268±0.081	0.190±0.02	0.486±0.098
Flavonoids (mg GAE/mg)	0.212±0.024	0.144±0.006	0.201±0.023

Each value represents Mean ± SD of three replicates.

Antioxidant activity

Figure 1. shows result of the methanol extract exhibited the highest radical scavenging activity with 68.82% at 100 µg/ml. The results revealed significant free radical scavenging activity of methanolic leaf extracts of *D. wallichii* on DPPH with IC_{50} value of 33.06 µg/ml. The positive control ascorbic acid showed the IC_{50} values of 33.09 µg/ml. According to these results, it was concluded that plant extracts from *D. wallichii* have potent antioxidant activity.

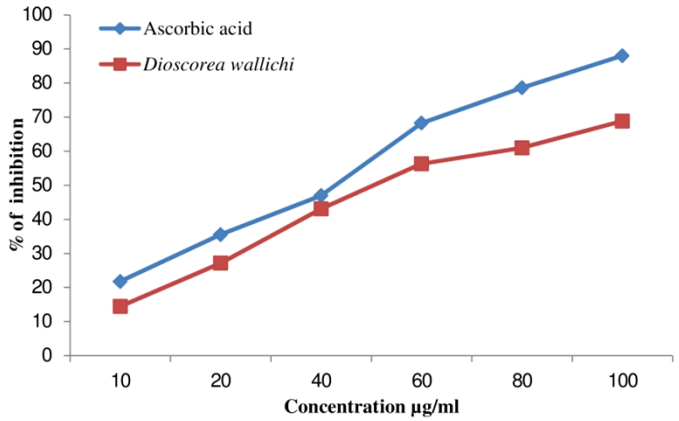

Fig. 1: DPPH free radical scavenging activity of crude methanol extracts *D. wallichii.*

Antimicrobial Activity

The antimicrobial activity of methanol, ethyl acetate and acetone leaf extracts at 4mg/ml concentrations of *D. wallichii* leaves extracts against some of the test organisms: Gram positive bacteria, Gram negative pathogenic bacteria and fungus are presented in Table 3. The methanol extract showed the potent inhibitory effect against *E. coli* (17.67mm) and *S. aureus* (18mm) compare to positive control. Methanol and ethyl acetate extract did not show any inhibitory effect against *C. albicans*. Acetone extract showed minimum antimicrobial activity against all test microbial organisms with zone of inhibition range of 9 -18.33mm.

Table: 3. Antimicrobial activity of three solvent extracts from the leaves of *D. wallichii*.

Microbial strain (species)	Solvent Extracts (Zone of (Inhibition) in mm)			
	Methanol	Ethyl acetate	Acetone	Gentamicin
Bacillus cereus	15.67±1.15	11.00±1.73	14.00±0.00	14.33±1.52
Klebsiella terrigena	8.18±1.16	10.00±0.00	15.00±1.41	25.00±0.82
Candida albicans	0.00±0.00	0.00±0.00	18.33±0.57	12.00±1.73
Pseudomonas aeruginosa	11.33±0.94	17.33±0.47	9.00±0.00	20.00±0.00
Staphylococcus aureus	18.00±1.52	9.00±0.00	12.00±1.52	16.30±0.57
Escherichia coli	17.67±0.47	10.00±0.00	17.00±0.00	17.33±0.47
Micrococcus mucilaginosus	15.66±0.57	9.66±0.57	12.66±0.57	16.00±1.73

Each value represents Mean ± SD of three replicates.

DISCUSSION

Phytochemical constituents in the plant samples are known to be biologically active compounds and they are responsible for different activities such as antioxidant, antimicrobial, antifungal, and anticancer (Hossain and Nagooru, 2011; Suresh and Nagarajan, 2009). Phenolics have antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutageneic, and antiinflammatory activities (Saidu *et al*., 2012; Sasikumar *et al*., 2010). These flavonoids also have antioxidant property as they inhibit oxidative and hydrolytic enzymes, have impact on radical scavenging, anti-inflammatory and anti-cancerous activity (Liu *et al*., 2008; Alsabri *et al*., 2013). The present study carried out phytochemical analysis on the medicinal plant extracts revealed the presence of medicinally active constituents. Previous report suggest the presence of following compounds: phenol, flavonoid, tannin and alkaloid in the *Dioscorea alata* (Das *et al*., 2014). Our phytochemical analysis of the leaves extracts of *Dioscorea wallichii* revealed similar results to previous findings. Phytochemical constituents such as alkaloids, glycosides, reducing sugar, flavonoids, tannins, saponins, and several other organic compounds are secondary metabolites of medicinal plants that serve as defense mechanism against many microorganisms and insects (Bonjar *et al*., 2004).

From the dose dependent response curve of DPPH radical scavenging activity of *D. wallichii*, it was observed that the methanol extract had higher radical scavenging activity. . In addition to this, the *D. wallichii* tested all the (acetone, ethyl acetae and methanol) extracts contains a significant amount of total phenols and flavonoids, which play a major role in controlling free radical formation. Our work on the DPPH radical scavenging activity of *D. wallichii* agrees with the findings of Rajalakshmi and Mohan 2013 on the DPPH radical scavenging of *D. tomentosa*.

These results are highly correlate with the previous reports on the medicinal plants *Dioscorea esculenta* (Thajunnisha Begum *et al*., 2013). Based on earlier reports, secondary metabolites found in plants are phenols and terpenoids which represent the main antimicrobial agents. Similarly, aromatic compounds such as phenolic acids, alkaloids and flavonoids have also been identified as antimicrobial agents (Siddiqui *et al*., 2009). The present study also showed the significant antimicrobial activities against the tested bacterial and fungal organisms due to the presence of high amount of phenol and flavonoid compounds present in the leaves of *D. wallichii*.

CONCLUSION

In conclusion, *D. wallichii* contain potential antimicrobial and phytochemical components that may be of great use for the development of pharmaceutics as a therapy against various diseases. This plant crude extracts could serve as potential sources of new antimicrobial and antioxidant agents.

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