Journal of Phytology 2011, 3(1): 26-32
Phytophysiology
ISSN: 2075-6240
Available Online: www.journal-phytology.com

REGULAR ARTICLE

ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF *ADIANTUM PEDATUM* L.

C.P. Chandrappa, C.B. Shilpashree, Karthik M.R, M. Govindappa* and T.S. Sadananda
Department of Biotechnology, Shridevi Institute of Engineering and Technology, SiraRoad, Tumkur 572106, Karnataka, India

SUMMARY

The four organic solvent extracts of *Adiantum pedatum* were tested for the potential antimicrobial activity against clinically important standard reference bacterial strains. The agar disk diffusion method was used to study the antibacterial activity of *Adiantum pedatum* extracts against 4 bacterial strains *Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Escherichia coli*. The phytochemical screening of extracts was carried out for major phytochemical derivatives in *Adiantum pedatum*. Two solvent extracts (acetone and ethyl acetate) showed inhibitory activity for *Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Escherichia coli*. The acetone and ethyl acetate extracts were found to be effective against *Staphylococcus aureus* than *Escherichia coli*. The phytochemical screening of extracts answered for the major derivative of terpenoids, cardiac glycosides and steroids. The antioxidant activity of the extracts of *Adiantum pedatum* was determined by the 1, 1-Diphenyl-2-picrylhydrazyl (DPPH), and ferrous reducing antioxidant property (FRAP) methods. Two extracts (acetone and ethyl acetate) showed antibacterial activity against the tested strains *Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Escherichia coli*. Of all, ethanol and hexane extracts did not showed any activity against tested strains. The results also indicate the presence of major phytochemical derivatives in the *Adiantum pedatum* extracts. Hence, the isolation and purification of therapeutic potential compounds from *Adiantum pedatum* could be used as an effective source against bacterial diseases in human and plants. The present study provides evidence that the extract of *Adiantum pedatum* is a potential source of natural antioxidants.

Key words: *Adiantum pedatum*, Phytochemicals, Antibacterial, Antioxidant activity

C.P. Chandrappa et al. Antibacterial and Antioxidant Activities of *Adiantum pedatum* L. J Phytol 3/1 (2011) 26-32.
*Corresponding Author, Email: dravidateja07@yahoo.co.in

1. Introduction

The genus *Adiantum pedatum* L. belongs to the Pteridaceae family, along with over 200 other species. Common name include 'maiden hair fern' or 'five fingers fern'. The Greek meaning of *Adiantum* is not wetting, which refers to the fact that even when the plant is immersed in water, it comes forth with dry leaves. It grows from Subarcitic North America into Deep South of the US. India is also native for *A.pedatum*. This species is usually grows about a foot tall, but can grow up to 2.5 feet tall. It prefers rich, moist soils that have a constant moisture supply. It also grows on a variety of rocks.

Borchardt *et al.* (2008) have tried *A. pedatum* leaves against *Staphylococcus aureus*. But no reports on other pathogens. The literature survey indicates that no reports are available from India regarding antibacterial and antioxidant properties of fern; *A. pedatum.* It is used to help relieve problems in inflammation of throat. It is also used for kidney stones, bladder gravel, and liver problems. It can be made into a tea to drink to help these problems. It is also used to get rid of worms from the body. The Native Americans used the maidenhair to make remedy that was used topically to stop the bleeding from a wound. The tea of fresh plant was used by the Greeks of old as expectorant for treating coughs. It has also been used to treat the painful and excessive

menstruation, as well as stimulating the onset and as a mild diuretic. It is also used by herbalists to treat sore throat, chronic nasal congestion, colds, severe coughs, bronchitis, whooping cough, excess mucus, upset stomach, urinary disorder, rheumatism, heartburn, alopecia (hair loss) and gallstone. In present study was aimed to examine the total phenolic content and phytochemical analysis of four solvent extracts of *A. pedatum* were screened for antibacterial and antioxidant properties using standard methods. The findings from this work may add to the overall value of the medicinal potential of the fern.

2. Materials and Methods

Plant collection

Adiantum pedatum were collected from Devarayanadurga forest, Tumkur district of Karnataka, India. Herbarium was prepared, identified by their vernacular names and authenticated with the herbarium at Department of Botany, University of Mysore, Mysore, Karnataka, India and Government Ayurvedic Medical College, Mysore, Karnataka, India. Plant materials were cleaned with deionized water and dried at shade for a week. Blotted plant materials were grounded and filtered using four layers of gauss cloth. The plant powder was stored in air tight container and maintained at 4°C until use.

Extract preparation

Ten g of powdered taken in a conical flask and subjected to extraction by using various solvents viz. acetone, ethyl acetate, ethanol and hexane separately. All the conical flasks were subjected to stirring by using magnetic stirrer for 2h and samples were subjected to centrifugation at 10,000 rpm at 4^{o}C for 10 min by using REMI cooling centrifuge. After centrifuge supernatant were collected in a separate conical flask and subjected to natural evaporation for 2 days. Thus the excess solvent was removed from the sample.

Test microorganisms

The 4 bacterial cultures of different strains, *Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa* and *E. coli* obtained from the authenticated stock culture of Department of Microbiology and Biotechnology, Bangalore University, Bangalore, Karnataka, India. Stock cultures were maintained at 4^{o} C on agar slants of nutrient media. Active cultures for experiment were prepared by transferring a loop full of microorganisms from the stock culture to eppendorff tubes which contained 1 ml of distilled water.

Screening for antibacterial activity

The disc diffusion method was used to screen the antimicrobial activity. The plates were prepared by pouring 15 ml of nutrient agar media in to sterile Petri plates. The plates were allowed to solidify for 5 min. And 1 µl of suspension culture was spread uniformly and the inoculum was allowed to dry for 5 min. Each extract was loaded (1.25 mg/ disc) on 6 mm sterile disc. The loaded disc was placed on the surface of media and allowed to diffuse for 5 min and the plates were kept for incubation at 37^{o} C for 24 h. At the end of the incubation inhibition zone formed around the disc were measured with scale in millimeter (Perumalsamy and Ignacimuthu, 2000).

Chemicals

All chemicals were of highest purity (≥ 99.0%),peptone, beef extract, NaCl, agar, HCl, $FeCl_2$, ethanol, hexane, acetone, ethyl acetate, dextrose, ammonia, H_2SO_4, ferric chloride, acetic anhydride, methanol, chloroform and glacial acetic acid were purchased from Vasa Scientifics, Bangalore, Karnataka, India. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2,4,6-tris (2-pyridyl)-S-triazine (TPTZ) was purchased from Aldrich Chemicals, Bangalore, Karnataka, India.

Phytochemical screening of the plant extract

Phytochemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedure to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

Test for Flavonoids

Five ml of the dilute ammonia solution was added to a portion of the aqueous filtrate of plant extract followed by addition of concentrated H_2SO_4. A yellow coloration

observed in extract indicated the presence of flavonoids. The yellow coloration disappeared on standing.

Test for Tannins

About 0.5g of dried powdered sample was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

Test for Steroids

Two ml of acetic anhydride was added to 0.5g ethanolic extract of sample with 2ml H_2SO_4. The colour changed from violet to blue or green in sample indicating the presence of steroids.

Test for Terpenoids

Five ml of plant extract was mixed in 2ml of chloroform, and concentrated sulphuric acid (3ml) was carefully added to form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of terpenoids.

Test for cardiac glycosides

One ml of plant extract was treated with 1ml of glacial acetic acid followed by treatment of 1 drop of 5% ethanolic ferric chloride solution. After this, added 1ml of concentrated H_2SO_4 poured along the sides of the test tubes. Appearance of a brownish ring between the two formed layers with lower acidic layer turning blue green upon standing indicated the presence of cardiac glycosides.

Antioxidant activity

In order to investigate the antioxidant properties of the examined extracts, ferric ion reducing antioxidant power (FRAP) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay were employed.

FRAP assay

FRAP reagent was freshly prepared by mixing 25 ml acetate buffer (300 mM, pH 3.6), 2.5 ml 2,4,6-tris (2-pyridyl)-S-triazine (TPTZ) solution (10 mM TPTZ in 40 mM/L HCl) and 2.5mL $FeCl_3$ (20 mM) water solution. Each sample (150 µL) (0.5 mg/ml) dissolved in all solvents separately was added in 4.5 ml of freshly prepared FRAP reagent and stirred and after 5 min, absorbance was measured at 593nm, using FRAP working solution as blank (Szollosi and Szollosi-Varga, 2002; Tomic et al., 2009). A calibration curve of ferrous sulfate (100-1000 µmol/L) was used and results were expressed in µmol Fe^{2+}/mg dry weight extract. The relative activity of the samples was compared to L-ascorbic acid.

DDPH radical assay

The effect of plant extracts on DPPH radical was estimated using the method of Liyana-Pathirana and Shahidi (2005). DPPH solution was freshly prepared by dissolve 24mg DPPH in 100ml methanol, stored at -20^{0}C before use. 150µl of sample (10µl sample + 140µl distilled water) is allowed to react with 2850µl of DPPH reagent (190µl reagent + 2660µl distilled water) for 24h in the dark condition. Absorbance was measured at 515nm. Standard curve is linear between 25 to 800µM ascorbic acid. Results expressed in µm AA/g fresh mass. Additional dilution needed if the DPPH value measured will over the linear range of the standard curve. Mix 10ml of stock solution in a solution of 45ml of methanol, to obtain an absorbance of 1.1±0.02 units at 517nm using spectrophotometer (Katalinic et al., 2006). All determinations were performed in triplicate. The percentage inhibition of DPPH radical by the samples was calculated according to formula of Yen and Duh (1994),

% inhibition= [{$Abs_{control}$-Abs_{sample}}/$Abs_{control}$] x 100,

Where $Abs_{control}$ is the absorbance of the DPPH radical+ ethyl acetate, Abs_{sample} is the absorbance of DPPH radical+ sample extract/standard.

Statistical Analysis

Percentage data were transformed to arcsine values and the analysis of variance was carried out (ANOVA). Means were compared for significance using Duncan's Multiple Range test (DMRT; P= 0.05)

3. Results

Antibacterial activity

The antibacterial activity of the extracts of *A. pedatum* plant is presented in Table 1. The acetone and ethyl acetate extracts showed significant antibacterial activity. The antibacterial activity of the acetone extract of *A. pedatum* is much higher than of ethyl acetate. Acetone and ethyl acetate extracts of

A. pedatum also have activity against all the organisms tested at different range. The other extracts (ethanol and hexane) were not shown antibacterial activity.

Table 1. Antibacterial activity of the crude extracts *Adiantum pedatum*

Organism(s)	Extracts and inhibition (cm)			
	Acetone	Ethyl acetate	Ethanol	Hexane
Staphylococcus aureus	2.4±0.03a	1.0±0.02c	NA	NA
Klebsiella pneumonia	1.25±0.03b	0.7±0.02d	NA	NA
Pseudomonas aeruginosa	1.5±0.02b	0.8±0.03c	NA	NA
E. coli	0.9±0.02c	0.7±0.02d	NA	NA

NA-No Activity,

Repeated the experiments three times for each replicates,

According to Duncan's Multiple Range Test (DMRT), values followed by different subscripts are significantly different at P<0.05, SE-standard error of the mean

Phytochemical analysis

The powdered were evaluated for qualitative and quantitative determination of major phyto constituents i.e. tannins, flavonoids, terpenoids, cardiac glycosides, steroids and phenols. The results of phytochemical analysis of *A. pedatum* plant are presented in Table 2. The ethyl extract yielded the presence of terpenoids, cardiac glycosides, steroids whereas the acetone also has all these phytochemical including phenols.

Table 2. Results of phytochemical analysis of *Adiantum pedatum*

Test	Acetone	Ethyl acetate	Ethanol	Hexane
Tannin	-	-	-	-
Flavonoids	-	-	-	-
Terpenoids	+	+	-	-
Cardiac glycosides	+	+	+	+
Steroids	+	+	+	+
Phenols	+	+	-	-

++= average, += minimum activity, -= No activity

Repeated the experiments three times for each replicates,

According to Duncan's Multiple Range Test (DMRT), values followed by different subscripts are significantly different at P≤0.05, SE-standard error of the mean.

Total phenol contents and antioxidant activity

Total Phenolic Content (TPC) was determined using the Folin- Ciocalteau reagent and expressed in terms of mg gallic acid equivalent (GAE)/ 100 ml extract. More TPC was observed in ethyl acetate extract (8.96) followed by acetone extract (8.72), ethyl alcohol extract (4.21) and hexane extract (4.05). The reducing ability of the extracts was in the range of 1113-3238 µm Fe (II)/mg (Table 3). The antioxidant potentials of the extracts of *A. pedatum* were estimated from their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II). The FRAP values for all the extracts were significantly lower than that of ascorbic acid but higher than that of BHT.

Table 3. FRAP assay to evaluate antioxidant property from different solvent extract of *A. pedatum*

Extract	FRAP	PAC
Acetone	2174±0.006^{b}	8.72±0.003^{a}
Ethyl acetate	3238±0.003^{a}	8.96± 0.007^{a}
Ethyl alcohol	1113±0.003^{c}	4.21±0.007^{b}
Hexane	1134±0.003^{c}	4.05±0.007^{b}

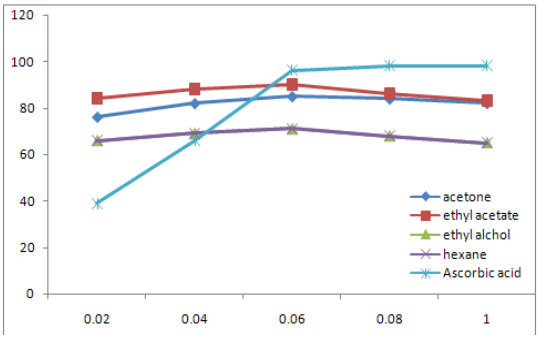
PAC: Phenolic Antioxidant Contents

Repeated the experiments three times for each replicates,

According to Duncan's Multiple Range Test (DMRT), values followed by different subscripts are significantly different at P≤0.05, SE-standard error of the mean.

The antioxidant activity of all the extracts as measured by the ability to scavenge DPPH free radicals was compared with the standards/ ascorbic acid and butylated hydroxyl toluene (BHT). It was observed that ethyl acetate extract of *A. pedatum* had higher activity than that of acetone, ethyl alcohol and hexane extracts. At a concentration of 0.1 mg/ml, the scavenging activity of ethyl acetate extract reached 90% and acetone, ethyl alcohol and hexane extracts reached 85%, 75% and 46% respectively. Though the DPPH radical scavenging abilities of the extracts were less than those of ascorbic acid (100%) and BHT (97.8%) at 0.1 mg/ml, the study showed that the extracts have the proton donating ability and could serve as free radical inhibitors or scavenging, acting possibly as primary antioxidants (Fig 1).

Figure 1. DPPH assay to determine antioxidant activity of different solvent extract of *A. pedatum*



4. Discussion

In the present study, the Phytochemical screening, antibacterial and antioxidant activities were performed with four different solvents, in that acetone and ethyl acetate extracts showed higher activity. The study was made against four bacteria using the standard disc diffusion method. The *A. pedatum* were rich in terpenoids, cardiac glycosides, steroids and phenols. These phytochemicals confer antimicrobial activity on total plant extracts. The acetone extract showed highest activity against all species bacteria tested. Acetone extract exhibited more activity against Gram-positive coccus bacteria, *Staphylococcus aureus* followed by ethyl acetate but other two extracts not shown any activity on bacteria tested. The inhibitory activity may be due to presence of phytochemicals in the extracts. Noticed phytochemicals also inhibited the free radicals in antioxidant activity method. The Terpenoids have been found to possess antioxidant and antimicrobial properties in various studies (Grassmann, 2005; Liu *et al*., 2009; Singh and Singh , 2003; Sauerwein *et al*.,1990; Iwu *et al*.,1990). The cardiac glycosides have been exhibited antioxidant and antimicrobial properties in various plant studies (Maneemegalai *et al*., 2010; Ajaiyeoba, 2002; Patil *et al*., 2010; Naveen Prasad *et al*., 2008; Ayoola *et al*., 2008; Prosper-Cabral *et al*., 2007; Anyasor *et al*., 2010). In various plant extracts, showed the presence of steroids and these have been inhibited the many bacteria and found to possess antioxidant potentials (Savage *et al*., 2002; Subisha and Subramoniam, 2005; Taleb - Contini *et al*., 2003; Mann *et al*.,2008; Geethalakshmi *et al*., 2010; Phadungkit *et al*., 2010; Shyamala and Vasantha., 2010). Presence of phenols in extract may explain its potent bioactivities as tannins are known to possess potent antioxidants and antibacterial activities (Memnune *et al*,.2009; Pereira et al., 2007; Oliveira et al., 2008; Gursoy et al., 2009; Turkoglu et al., 2010; Roman *et al*., 2010; Gulumser *et al*., 2010).

The present investigation has shown that acetone and ethyl acetate extract have active phytochemicals (terpenoids, cardiac glycosides, steroids and phenols) which are able to inhibit the pathogenic bacteria. The strong antioxidant activity was confirmed in both he extracts. Both antimicrobial and antioxidant activity may be due to strong occurrence of polyphenoloic compound such as terpenoids, cardiac glycosides, steroids and phenols. These findings provide scientific evidence to support traditional uses and indicate a promising potential for the development an antimicrobial and antioxidant drug from *A.pedatum* plant. This medicinal plant by *in vitro* results appears as interesting and promising and may be effective as potential sources of novel antimicrobial antioxidant drugs.

Acknowledgement

The authors are thank Dr M.R. Hulinaykar, Managing Trustee, SCT, Tumkur and Dr M.A. Venkatesh, Principal of

SIET for their help and suggestions for present investigation.

References

Ajaiyeoba, E.O., Phytochemical and antibacterial properties of *Parkia biglobosa* and *bicolor* leaf Extracts, *African Journal. Biomed. Res*., 2002, 5, P. 125 – 129.

Anyasor, G.N., Ogunwenmo, K.O., Oyelana, O.A. and Akpofunure, B.E., Phytochemical constituents and antioxidant activities of aqueous and methanol stem extracts of *Costus afer* Ker Gawl. (Costaceae), *African Journal of Biotechnology,* 2010, 9 (31), P. 4880-4884.

Ayoola, G.A., Coker, H.A.B., Adesegun, S.A., Adepoju-Bello, A.A., Obaweya, K., Ezennia, E. C. and Atangbayila, T.O., Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria, *Tropical Journal of Pharmaceutical Research,* 2008, 7(3), P. 1019-1024

Borchardt, J.R., Donald, L.W., Craig, C., Sheaffer, K. L., Kauppi, Fulcher, R.G., Nancy, J.E., David, D.B. and Russell, F.B., Antioxidant and antimicrobial activity of seed from plants of the Mississippi river basin, *Journal of Medicinal Plants Research*., 2008, 2(4), P. 081–093.

Geethalakshmi, R., Sarada, D.V. L. and Marimuthu, P., Evaluation of antimicrobial and antioxidant potentials of *Trianthema decandra* L., *Asian J. Biotechnol*., 2010, 2, P. 225-231.

Grassmann, J., Terpenoids as plant antioxidants, *Vitam Horm*., 2005, 72, P.505-35.

Gulumser, A., Dogan, N.M., Duru, M.E. and Kivrak, I., Phenolic profiles, antimicrobial and antioxidant activity of the various extracts of *Crocus* species in Anatolia, *African Journal of Microbiology Research,* 2010, 4(11), P.1154-1161.

Gursoy, N. and Tepe, B., Determination of the antimicrobial and antioxidative properties and total phenolics of two "endemic" Lamiaceae species from Turkey: *Ballota rotundifolia* L. and *Teucrium chamaedrys* C. Koch., *Plant Foods Hum Nutr*., 2009, 64(2), P. 135-140.

Harborne, J.B., Phytochemical methods, London. Chapman and Hall, Ltd. 1973, P. 49-188.

Iwu, M., Ezeugwu, C., Okunji, C., Sanson, D. R. and Tempesta, M.S., Antimicrobial activity and terpenoids of the essential oil of *Hyptis suaveolens*, *Pharmaceutical Biology*, 1990, 28(1), P. 73-76.

Katalinic, V., Milos, M., Kulisic, T. and Jukic, M., Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols, *Food Chem*., 2006, 94, P. 550–557.

Liu, X.P., Luan, J.J. and Goldring, C.E., Comparison of the antioxidant activity amongst *Gingko biloba* extract and its main components, *Zhong Yao Cai*., 2009, 32(5), P. 736-740.

Liyana-Pathiranan, Chandrika, M. and Shahidi, F., Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions, *J.Agri.Food Chem*., 2005, 53, P. 2433.

Maneemegalai, S. and Naveen, T., Evaluation of antibacterial activity of flower extracts of *Cassia auriculata, Ethnobotanical Leaflets*, 2010, 14, P. 8-20.

Mann, A., Yahaya, Y., Banso, A. and John, F., Phytochemical and antimicrobial activity of *Terminalia avicennioides* extracts against some bacteria pathogens associated with patients suffering from complicated respiratory tract diseases, *Journal of Medicinal Plants Research,* 2008, 2(5), P. 094-097.

Memnune, S., Yildiz, H., Gungor, N., Cetin, B., Eser, Z. and Ercisli, S., Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants, *Pak. J. Pharm. Science*., 2009, 22(1), P. 102-106.

Naveen Prasad, R., Viswanathan, S., Renuka, J.D., Vijayashree, N., Swetha, V.C., Archana, B.R., Parathasarathy, N. and Rajkumar, J., Preliminary phytochemical screening and antimicrobial activity of *Samanea saman*, *Journal of Medicinal Plants Research,* 2008, 2(10), P. 268-270.

Oliveira, I., Sousa, A., Ferreira, I.C., Bento, A., Estevinho, L., Pereira, J.A., Total phenols, antioxidant potential and antimicrobial activity of walnut (*Juglans*

regia L.) green husks, *Food ChemToxicol*., 2008, 46(7), P. 2326-2331.

Patil, U.H., Gaikwad, D.K. and Chavan, P.D., Antibacterial activity of stem bark of *Boswellia serrata* (Roxb.), *Bioinfolet,* 2010, 7.2.

Pereira, J.A., Oliveira, I., Sousa, A., Valentao, P., Andrade, P.B., Ferreira, I.C., Ferreres, F., Bento, A., Seabra, R., Estevinho, L., Walnut (*Juglans regia* L.) leaves: Phenolic compounds, antibacterial activity and antioxidant potential of different cultivars, *Food Chem Toxicol.*, 2007, 45(11), 2287-95.

Perumalsamy, R. and Ignacimuthu, S., Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats of India, *J of Ethnopharmacology,* 2000,69, P.63-71.

Prosper-Cabral, N.B., Agbor, G.A., Oben, J.E. and Ngogang, J.Y., Phytochemical studies and antioxidant properties of four medicinal plants used in Cameroon, *Afr J Tradit Complement Altern Med*., 2007, 4(4), P. 495–500.

Phadungkit, M., Rattarom, R. and Rattana, S., Phytochemical screening, antioxidant, antibacterial and cytotoxic activities of *Knema angustifolia* extract, *Journal of Medicinal Plants Research,* 2010, 4(13), P. 1269-1272.

Roman, M., Hradkova, I., Filip, V. and Smidrkal, J., Antimicrobial and antioxidant properties of phenolic acids alkyl esters, *Czech J. Food Sci.*, 2010, 28(4), P. 275–279.

Sauerwein, M. and Becker, H., Growth, terpenoid production and antibacterial activity of an *in vitro* culture of the liverwort *Fossombronia pusilla*., *Planta Med*., 1990, 56(4), P. 364-367.

Savage, P.B., Li, C., Taotafa, U., Ding, B. and Guan, Q., Antibacterial properties of cationic steroid antibiotics, *FEMS Microbiol Lett*., 2002, 217(1), P. 1-7.

Shyamala, G.S. and Vasantha, K., Phytochemical screening and antibacterial activity of *Syzygium cumini* L. (Myrtaceae) leaves extracts, *International Journal of PharmTech Research,* 2010, 2(2), P. 1569-1573.

Singh, B. and Singh, S., Antimicrobial activity of terpenoids from *Trichodesma amplexicaule* Roth., *Phytother Res*., 2003, 17(7), P. 814-816

Sofawara, A., Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria. 1993, P. 289.

Subhisha, A. and Subramoniam, A., Antifungal activities of a steroid from *Pallavicinia lyellii, Indian Journal of Pharmacology*, 2005, 37(5), P. 304-308.

Szőllősi, R. and Szőllősi Varga, I., Total antioxidant power in some species of Labiatae (Adaptation of FRAP method). *Acta Biol Szeged.,* 2002, 46, P. 125–127.

Taleb-Contini, S.H., Salvador, M.J., Watanabe, E., Ito, I.Y. and Rodrigues de, O.D.C., Antimicrobial activity of flavonoids and steroids isolated from two *Chromolaena* species, *Revista Brasileira de Ciencias Farmaceuticas,* 2003, 39(4), P. 403-408.

Tomić, A., Petrović, S., Pavlović, M., Trajkovski, B., Milenković, M., Vučićević, D. and Niketić, M., Antimicrobial and antioxidant properties of methanol extracts of two *Athamanta turbith* subspecies, Pharmaceutical Biology, 2009, 47(4), P. 314–319.

Trease, G.E. and Evans, W.C., Pharmacognsy. 11th edn. Brailliar Tiridel Can. Macmillian publishers, 1989.

Turkoglu, S., Turkoglu, I., Kahyaoglu, M. and Celık, S., Determination of antimicrobial and antioxidant activities of Turkish endemic *Ajuga chamaepitys* L. Schreber subsp. *Euphratica* P.H. Davis (Lamiaceae), *Journal of Medicinal Plants Research,* 2010, 4(3), 1260-1268

Yen, G.C. and Duh, P.D., Scavenging effect of methanolic extracts of peanut hulls on free-radical and active oxygen species, *J. Agric. Food Chem.*, 1994, 42, P.629-632.