

## DETERMINATION OF DIFFERENT ANTIOXIDANT COMPOUNDS FROM THE MANGROVE PLANTS OF *PANDANUS FOETIDUS* AND *AVICENNIA OFFICINALIS*

Farjana Islam Aovi, Md. Mominur Rahman

Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University, Dhaka, Bangladesh

[farjana.pharm@diu.edu.bd](mailto:farjana.pharm@diu.edu.bd)

### Abstract

Recently, medicinal plants have grabbed attention worldwide in the field of neurosciences for therapeutic intervention. The aim of the study was to identify and measure the total phenolic content (TPC), total flavonoids content (TFC), tannin content (TPC) and total antioxidant capacity (TAC) of the 3 ethanolic extracts. Three ethanolic extracts from two mangrove plants were subjected to the investigation for identifying different antioxidant compounds. For the tests, we used the leaves and aerial root extract from *Pandanus foetidus* and leaves from *Avicennia officinalis*. These two are mangroves plants with the evidence of presence of prominent antioxidant properties. The highest phenolic and tannin content were measured about 269mg GAE/gm dry extract and about 212mg AAE/gm dry extract of *P. foetidus* leaves extract. Total flavonoid content was high in *A. officinalis* leaves extract which was about 176mg QE/gm dry extract. Aerial root extract of *P. foetidus* showed lower TPC, TFC and TTC in compare with other extracts. The highest TAC were identified about 212mg AAE/gm dry extract which actually support the highest TAC and TTC in *P. foetidus* leaves extract. The findings of this study indicate that the different antioxidant compounds in this study may be considered interesting candidates for future research.

**Keywords:** Total phenolic content, total flavonoid content, total tannin content, total antioxidant capacity

## Introduction

Mangrove plants are specialized plants that grow in the tidal coasts of tropic and subtropics regions of the world. Their unique ecology and traditional medicinal usages of mangrove plants have concerned the consideration of researchers over the years, and as a result, information on biological activity of mangrove plants have increased meaningfully in recent centuries [1]. Mangroves are generally initiated along the coastlines of tropical and subtropical regions, usually between 25° N and 25° S latitude, throughout the world. As an exception to these, mangroves remain originated as far south as New Zealand and as far as north as Japan [2]. Mangroves once enclosed  $\frac{3}{4}$  of the world's tropical seashores, often in conjunction with the coral reefs. Asia comprises most of the world's mangroves with 46%, trailed by America with 35 % and Africa with 17% [3]. Particular environmental factors such as temperature, deep sea current, rainfall, salinity stress, wave action, sedimentation, fresh water flow etc. regulate the occurrence and development of mangroves in the native area. The Sundarban is the single biggest continuous mangrove forest in the world and has been renowned as an international important Ramsar Wetland Site and acknowledged as a World Heritage Site (WHS) by the UNESCO in 1997. The Sundarbans delta bounces across coastal India and Bangladesh, over the northern portion of the Bay of Bengal. It is positioned in the south west of Bangladesh and the south eastern portion of the State of West Bengal in India [4].

About 62% of the Sundarbans forest is in Bangladesh and the rest in India. According to two reputable scientific studies, mangroves comprise about 16-24 families and 54-75 species [5] respectively. The utmost diversity of mangrove species happens in Southeast Asia. For example, there are only twelve mangrove species in the New World and only four species of mangroves exist lengthways the coasts of the southern USA [6]. Floral diversity of Sundarban mangrove forests is very rich compared to other mangroves in the world. HENING (1892) recorded 69 species under 34 families in the whole of Sundarban (Bangladesh and India) territory. Karim et al. [7] stated 123 plant species belonging to 22 families on behalf of 30 genera in SMF in Bangladesh. HOSSAIN [8] reported 44 undergrowth classes of Sundarban

mangrove forest. As a substance of fact, SMF has not yet been thoroughly discovered floristically, but for a few sporadic trips completed by the above-mentioned scientists and teams of Bangladesh National Herbarium, IUCN, UNDP, ODA and FAO. The Sundarban forest is conquered mostly by three major tree species, viz. *Heritiera fomes*, *Excoecaria agallocha* and *Ceriops decandra* [9]. Mangrove and mangrove associates cover biologically active antiviral, antibacterial and antifungal compounds [10]. They deliver a rich source of steroids, triterpenes, saponins, flavonoids, alkaloids and tannins [11]. Peninsular India includes about 6700 km<sup>2</sup> of mangrove forest consisting of two types of territories: the deltaic east coast (Bay of Bengal) and backwater-estuarine west coast (Arabian Sea) [12,13]. Although some studies are obtainable on the mangrove mycota of the Indian peninsula, few studies have dealt with fungal richness and diversity [14-18]. In contrast, there is no information on the species richness and diversity of advanced fungi on specific mangrove woody fragments of southwest coast of India [19].

*Pandanus foetidus* Roxb. (Pandanaceae), locally known as kewa kata, Keora, Keurikanta or Kewakanta, a communal hedge-plant with no proper stem, grows through Bangladesh, mainly in the coastal region of the mangrove forest, Sundarban [20] and Chittagong. Leaves of this plant remain used in leprosy, small pox, syphilis, scabies besides heart and brain diseases [21] Leaves and spadix are similarly used in diabetes [22]. Methanol extract of *P. foetidus* leaves was established to show potential depressant result on the central nervous system by intrusive the cortical function in mice. Essential oil of *P. foetidus* is also used as perfumery as well as medicinal sources [23].

*Avicennia officinalis* (L.) is dominant in most of the regions of Indian coast. This is a tall (25 m) and thick (1 m in diameter) evergreen tree with abundant pneumatophores rising above soil from the underground cable root. These pneumatophores are very unique structures because they bear lenticels for gaseous exchange enabling the mangroves to survive in the hypoxic water logged environment. Pneumatophores are ideal substratum for the epibiosis and are believed to bear many more cryptic species which are unknown to science [24-26].

Antioxidants are compounds that inhibit oxidation. Oxidation is a chemical reaction that can yield free radicals, thereby principal to chain reactions that may harm the cells of organisms. Antioxidants such as thiols or ascorbic acid (vitamin C) dismiss these chain reactions. To balance the oxidative state, plants and animals continue complex systems of overlapping antioxidants, such as glutathione and enzymes formed internally, or the dietary antioxidants vitamin C, and vitamin E [27]. Antioxidant dietary supplements have not been exposed to recover health in humans, or to be operative at preventing disease [28]. Supplements of beta-carotene, vitamin A, and vitamin E have no positive effect on mortality rate [29,30] or cancer risk [31,32]. Additionally, supplementation with selenium or vitamin E do not decrease the risk of cardiovascular disease [33, 34]. The aim of the study was to measure various antioxidant content in three different extracts.

#### Methods and Materials

**Chemicals and Reagents:** Ethanol, Distilled water, Folin-Ciocalteu (FC) reagent, Gallic acid,  $\text{Na}_2\text{CO}_3$ ,  $\text{H}_2\text{SO}_4$  (0.6M), Ascorbic acid,  $\text{Na}_3\text{PO}_4$ , Ammonium Molybdate Ethanol, Gallic acid (as positive control), Methanol, Quercetin, 5 % Sodium Nitrite ( $\text{NaNO}_2$ ) solution, 10% Aluminium Chloride ( $\text{AlCl}_3$ ) solution, 1M Sodium Hydroxide (NaOH) solution

#### Collection of plants:

Selected plants were collected directly from the Sundarban, Koromjal range Mongla, Khulna, Bangladesh on 25 November, 2014 and were identified at Bangladesh National Herbarium, Mirpur, Dhaka, where vouchers have been issued against each plant species (voucher specimen no. 37134 DACB issued for *P. foetidus* & 37137 DACB for *A. officinalis*)

#### Preparation of plant extract:

The leaves and aerial root were cut into small pieces and the shade dried materials were grinded into fine powder which had been made completely devoid of any water content. Then about 150gm of powdered materials were macerated into sufficient ethanol for 10 days. After filtration through Whatman filter paper, the filtrates were concentrated at 40°C with a rotary evaporation. The concentrated extracts were then preserved in individual containers in refrigerator with demakation.

#### Determination of total phenolic content:

The Folin–Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventos, 1999) [35] was used to determine total phenolic content. Each ethanolic sample (5 g) was diluted to 50 ml with distilled water and filtered through Whatman No. 1 paper. This solution (0.5 ml) was then mixed with 2.5 ml of 0.2 N Folin–Ciocalteu reagent for 5 min and 2 ml of 75 g/l sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) was then added. After incubation at room temperature Absorbance of the mixtures was measured at 750 nm (UV-Visible Ultraspec 2000 spectrophotometer, England). Gallic acid (0–200mg/l) was used as standard to produce the calibration curve. The mean of three readings was used and the total phenolic content was expressed in mg of gallic acid equivalents (GAE)/100 g of extract.

#### Determination of Total flavonoid contents:

The total flavonoid content was determined using the Dowd method as adapted by Arvouet-Grand, et al., 1994 [36]. Briefly, 5 ml of 2% aluminium trichloride ( $\text{AlCl}_3$ ) (Labosi, Paris, France) in methanol (Fluka Chemie, Switserland) was mixed with the same volume of a honey solution (0.01 or 0.02 mg/ml). The absorbance was measured at 510 nm against the blank by using spectrophotometer (double beam Shimadzu UV/ visible spectrophotometer (Model 1800, Japan and were taken after 10 min against a blank sample consisting of a 5 ml honey solution with 5 ml methanol without  $\text{AlCl}_3$ . The total flavonoid content was determined using a standard curve with quercetin (Sigma–Aldrich Chemie, Steinheim, Germany) (0–50 mg/l) as the standard. The mean of three readings was used and expressed as mg of quercetin equivalents (QE)/100 g of extract.

#### Determination of total tannin contents:

The total tannin content was determined using the Folin-Ciocalteu phenol reagents as reported by Amorim et al., 2008. [37] Briefly 0.1 ml of the sample extract is added with 7.5 ml of distilled water and then added 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35% sodium carbonate solution and dilute to 10 ml with distilled water. The mixture was shaken well kept at room temperature for 30 min and absorbance was measured at 725 nm with a double beam UV/ Visible spectrophotometer double beam Shimadzu UV/ visible spectrophotometer (Model 1800, Japan). The total tannin content was determined as mg of Gallic acid equivalent per gram

of dry extract obtained from a standard Gallic acid calibration curve. The mean of three readings was used and the total phenolic content was expressed in mg of gallic acid equivalents (GAE)/100 g of extract.

#### **Estimation of total antioxidant capacity:**

The total antioxidant capacity of the extracts was determined by phosphomolybdate method by the method of Prieto *et al.*, 1999. [38] 0.3 ml of extract and ascorbic acid used as standard and blank (ethanol) were combined with 3 ml of reagent mixture separately and incubated at 95°C for 90 minutes. After cooling to room temperature, the absorbance of each sample was measured at 695 nm against the blank. The total antioxidant content was determined using a standard curve by phosphomolybdate method using ascorbic acid as a standard [39]. The mean of three readings was used and expressed as mg of Ascorbic Acid Equivalent (AAE)/100 g of extract.

**Statistical Analysis** All the assays were performed in triplicate and the results are expressed by the mean values and standard deviation. Significant differences for multiple comparisons were determined by one-way analysis of variance (ANOVA) followed by Dunnet's test. The analysis was carried out using Microsoft® Office Excel (Microsoft®, USA), where values of  $p \leq 0.05$  were considered statistically significant.

#### **Results and Discussion**

Medicinal plants are good resource of commercial drugs for the production or in the improvement of lead compounds, it has been revived. Most of the drugs which are used for depression affect the quality life of sick people. Oppositely, herbal medicines have less toxicity, good absorption and have a lower side effect profile. That's why, this has been used since very old times [40]. So it is necessary to create efforts to represent the new medicinal plants for production of cheaper and less toxic drugs.

Free radicals are often generated as by product of biological reactions or from exogenous factors. Antioxidants can prevent many diseases associated with excess free radical by reducing their amount in the body.

The result of the present study showed that the extract of *A. officinalis* leaves and aerial root extract from *Pandanus foetidus* contain some antioxidant

capacity but *P. foetidus* leaves extract had higher total antioxidant capacity than other two extracts. In case of total tannin capacity, *P. foetidus* leaves extract also contain the highest amount. The extract of *A. officinalis* leaves which contain high amount of flavonoid compounds exhibited good antioxidant activity may be of the design of further studies to unravel novel treatment strategies for disorders associated with free radicals induced tissue damage. The result showed that the extract of *P. foetidus* leaves contain high amount of phenolic compounds which exhibited good antioxidant activity compared with other two extract. Aerial root extract of *P. foetidus* showed lower TPC, TFC and TTC in compare with other extracts. The highest phenolic and tannin content were measured about by dry extract of *P. foetidus* leaves. [Table 1-3 and Figure 1-7]

Now it can be concluded on the basis of results obtained from investigation that the plant may be useful as antioxidant agent. But our work was only preliminary effort. It will require additional detailed advanced investigation.

#### **Conclusions**

From the thousands of years, nature is giving us medicinal gift which act as natural source of modern drugs. *A. officinalis* and *P. foetidus* which contain so many pharmacological activities. To examine the pharmacological activity, different antioxidant activity was evaluated by determination of total phenolic content, total antioxidant capacity, total total tannin content and total flavonoid content. After observing the results of recent study, it can be said that the leaves and aerial root extract from *Pandanus foetidus* and extract of the leaves from *Avicennia officinalis* showed significant antioxidant activity.

So it is clear that our experimental plant possesses antioxidant activity and requires further investigation to identify active compounds.

#### **Acknowledgments**

The authors are grateful to Department of Pharmacy, Daffodil International University to give permission and all sorts of supports to conduct the research.

#### **References**

1. Shilpi JA, Islam ME, Billah M, Islam KM, Sabrin F, Uddin SJ, Nahar L, Sarker SD. Antinociceptive, anti-inflammatory, and antipyretic activity of mangrove



- plants: A mini review. *Advances in pharmacological sciences*. 2012; 2012.
2. Choudhury JK. Sustainable management of coastal mangrove forest development and social needs. In *Proceedings of the XI World Forestry Congress 1997* (Vol. 6, pp. 266-286).
  3. MAP (Mangrove Action Project) (1990): *Mangrove Ecosystems*.
  4. Acharya G, Kamal D. Fisheries. *Mangrove of the Sundarbans, Volume Two: Bangladesh*, Hussain, Z. and Acharya, G.(eds.). IUCN-The World Conservation Union, Switzerland. 1994.
  5. Gilman E, Ellison J, Coleman R. Assessment of mangrove response to projected relative sea-level rise and recent historical reconstruction of shoreline position. *Environmental monitoring and assessment*. 2007;124 (1-3):105-30.
  6. Alfredo Q. Mangrove Action Project (background paper). Ramsar web site, Rue Mauverney. 1997;28.
  7. KARIM, A. Vegetation. In: *Mangroves of the Sundarbans. Volume two: Bangladesh*. Edited by HUSSAIN, Z. & G. ACHARYA (1994). IUCN, Bangkok, Thailand: 43-75.
  8. Hossain AB. The undergrowth species of Sundarban mangrove forest ecosystem (Bangladesh). The final report on Sundarban Biodiversity Conservation Project, IUCN, Dhaka, Bangladesh (unpublished). 2003.
  9. Rashid SH, Böcker R, Hossain AB, Khan SA. Undergrowth species diversity of Sundarban mangrove forest (Bangladesh) in relation to salinity. *Ber. Inst. Landschafts-Pflanzenökologie Univ. Hohenheim*. 2008(17):41-56.
  10. Bandaranayake WM. Traditional and medicinal uses of mangroves. *Mangroves and Salt Marshes*. 1998;2:133-148.
  11. Bandaranayake WM. Survey of mangrove plants from Northern Australia for phytochemical constituents and uv-absorbing compounds. *Current Topics in Phytochemistry (Life Science Advances)* 1995;14:69-78
  12. Natarajan R. The mangroves: a natural, renewable economic resource needs conservation. An anthology of Indian mangroves (A commemorative volume on the Golden Jubilee of India's independence).1998:1-6.
  13. Kathiresan K. Global biodiversity of mangroves in relation to India. In *Proceedings of UNU International Training Workshop on Methodologies for Assessment of Biodiversity in Estuaries, Mangroves and Coastal Waters*. Centre of Advanced Study in Marine Biology, Annamalai University, India 1999 (pp. 105-122).
  14. Borse, B.D. Frequency of occurrence of marine fungi from Maharashtra coast, India. *Indian Journal of Marine Sciences* 1988;17: 165-167.
  15. Borse, B.D., Kelkar, D.J. and Patil, A.C. Frequency of occurrence of marine fungi from Pirotan Island (Gujarat), India. *Geobios*. 2000;27: 145-148.
  16. Patil, K.B. and Borse, B.D. Studies on higher marine fungi from Gujarat coast (India). *Geobios*.2001; 28: 41-44.
  17. Ravikumar, D.R. and Vittal, B.P.R. Fungal diversity on decomposing biomass of mangrove plant *Rhizophora* in Pichavaram estuary, east coast of India. *Indian Journal of Marine Sciences*.1996;25: 142-144
  18. Sarma, V.V. and Vittal, B.P.R. Biodiversity of mangrove fungi on different substrata of *Rhizophora apiculata* and *Avicennia* spp. from Godavari and Krishna deltas, east coast of India. *Fungal Diversity*. 2000;5: 23-41.
  19. Maria GL, Sridhar KR. Diversity of filamentous fungi on woody litter of five mangrove plant species from the southwest coast of India. *Fungal Diversity*. 2003;14(14):109-26.
  20. Chaffey DR. A glossary of vernacular plant names and a field key to the trees. South-west Ethiopian forest inventory project. Surbiton, Land Resources Development Centre. 1978:45.
  21. Joshi, S.G. *Medicinal Plants*. Oxford and IBH Publishing Co. Ltd, India, 2000.
  22. Yusuf M, Chowdhury JU, Wahab MA, Begum J. *Medicinal plants of Bangladesh*. Bangladesh Council of Scientific and Industrial Research, Dhaka, Bangladesh. 1994;192.
  23. Uddin SJ, Shilpi JA, Delazar A, Nahar L, Sarker SD. Free radical scavenging activity of some Bangladeshi plant extracts. *Oriental Pharmacy and Experimental Medicine*. 2004;4(3):187-95.
  24. Procheş Ş, Warren M, McGeoch MA, Marshall DJ. Spatial scaling and transition in pneumatophore arthropod communities. *Ecography*. 2010;33(1):128-36.
  25. Sahoo G., Suchiang S.R., Ansari Z.A. MeiofaunaMangrove interaction: a pilot study from a tropical mangrove habitat — *Cah. Biol. Mar*. 2013;54: 349-358

26. Larsen K., Sahoo G., Ansari Z.A. Description of a New Mangrove Root Dwelling Species of Teleotanis (Crustacea: Peracarida: Tanaidacea) from India, with a Key to Teleotanidae Species Diversity. 2013;18: 237-243
27. Dabelstein W, Reglitzky A, Schütze A, Reders K. Automotive fuels. Ullmann's Encyclopedia of Industrial Chemistry. 2007.
28. "Antioxidants: In Depth". NCCIH. Retrieved 20 June 2018.
29. Bjelakovic G, Nikolova D, Gluud C. Meta-regression analyses, meta-analyses, and trial sequential analyses of the effects of supplementation with beta-carotene, vitamin A, and vitamin E singly or in different combinations on all-cause mortality: do we have evidence for lack of harm?. PLoS one. 2013;8(9):e74558.
30. L Abner E, A Schmitt F, S Mendiondo M, L Marcum J, J Kryscio R. Vitamin E and all-cause mortality: a meta-analysis. Current aging science. 2011;4(2):158-70.
31. Caraballoso M, Sacristan M, Serra C, Cosp XB. Drugs for preventing lung cancer in healthy people. Cochrane Database of Systematic Reviews. 2003(2).
32. Jiang L, Yang KH, Tian JH, Guan QL, Yao N, Cao N, Mi DH, Wu J, Ma B, Yang SH. Efficacy of antioxidant vitamins and selenium supplement in prostate cancer prevention: a meta-analysis of randomized controlled trials. Nutrition and cancer. 2010;62(6):719-27.
33. Rees K, Hartley L, Flowers N, Clarke A, Hooper L, Thorogood M, Stranges S. 'Mediterranean' dietary pattern for the primary prevention of cardiovascular disease. Cochrane Database of Systematic Reviews. 2013(8).
34. Shekelle PG, Morton SC, Jungvig LK, Udani J, Spar M, Tu W, Suttorp MJ, Coulter I, Newberry SJ, Hardy M. Effect of supplemental vitamin E for the prevention and treatment of cardiovascular disease. Journal of general internal medicine. 2004;19(4):380-9.
35. Singleton VL, Orthofer R, Lamuela-Raventós RM. [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In Methods in enzymology 1999 (Vol. 299, pp. 152-178). Academic press.
36. Arvouet-Grand A, Vennat B, Pourrat A, Legret P. Standardization of propolis extract and identification of principal constituents. Journal de pharmacie de Belgique. 1994; 49(6):462-8.
37. Amorim EL, Nascimento JE, Monteiro JM, Peixoto Sobrinho TJ, Araújo TA, Albuquerque UP. A simple and accurate procedure for the determination of tannin and flavonoid levels and some applications in ethnobotany and ethnopharmacology. Functional Ecosystems and Communities. 2008;2(1):88-94.
38. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical biochemistry. 1999;269(2):337-41.
39. Umamaheswari M, Chatterjee TK. In vitro antioxidant activities of the fractions of *Coccinia grandis* L. leaf extract. African Journal of Traditional, Complementary and Alternative Medicines. 2008;5(1):61-73.
40. Li RW, Myers SP, Leach DN, Lin GD, Leach G. A cross-cultural study: anti-inflammatory activity of Australian and Chinese plants. Journal of Ethnopharmacology. 2003;85(1):25-32.

**Table 1.** Amount of TPC, TFC, TTC and TAC in *P. foetidus* leaves extract

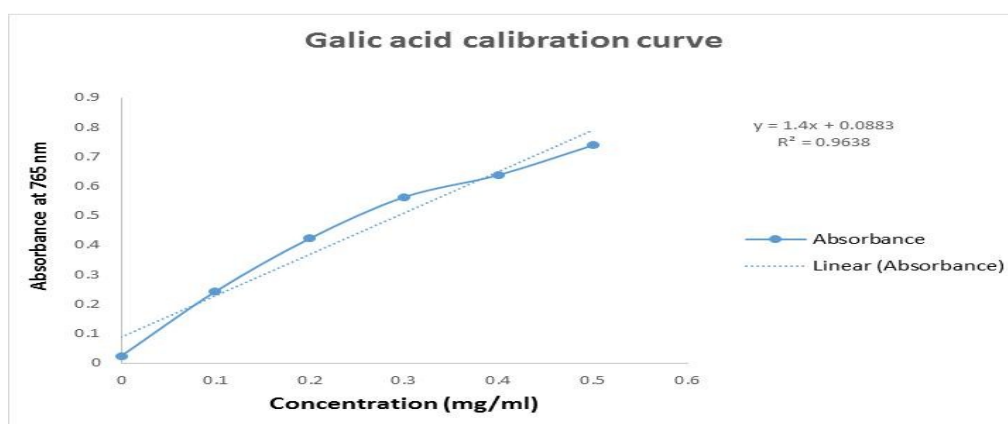
TPC (mg GAE/gm of dry extract)	TFC (mg QE/gm dry extract)	TTC (mg GAE/gm of dry extract)	TAC (mg AAE/gm dry extract)
269.98±0.002	72.02±0.002121	70.01±0.002	212.90±0.63

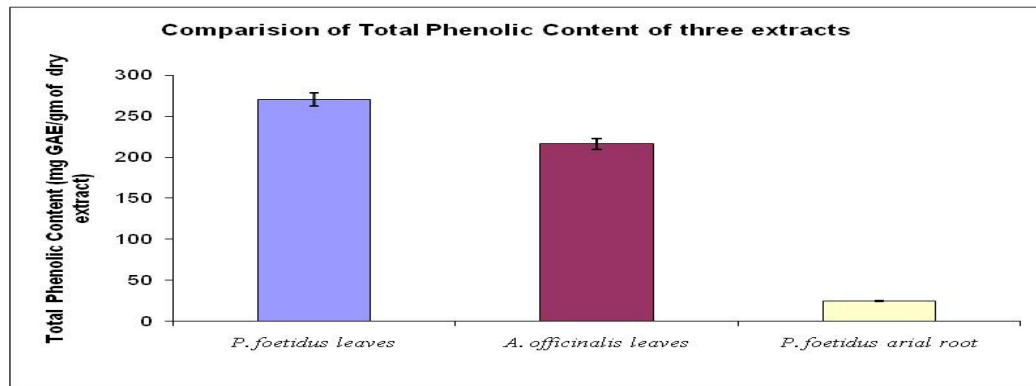
**Table 2.** Amount of TPC, TFC, TTC and TAC in *P. foetidus* arial root extract

TPC (mg GAE/gm of dry extract)	TFC (mg QE/gm dry extract)	TTC (mg GAE/gm of dry extract)	TAC (mg AAE/gm dry extract)
25.14± 0.002	54.67±0.002121	39.94±0.002	48.16±0.63

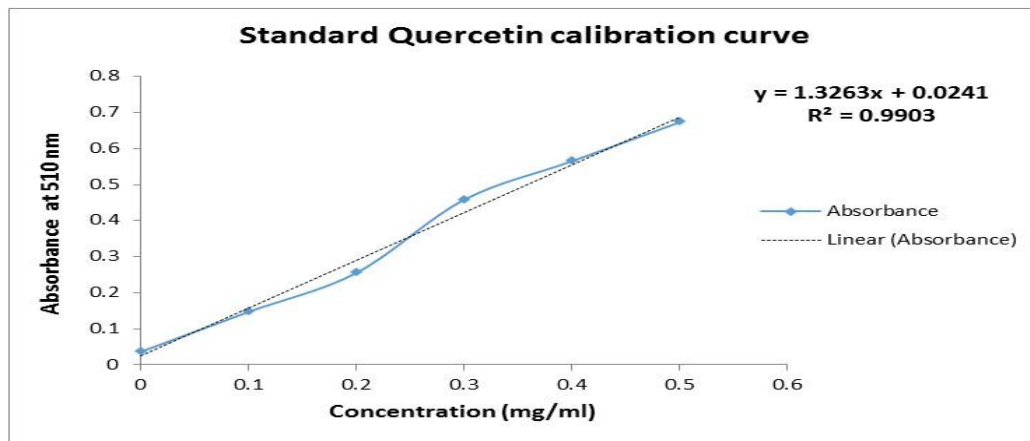
**Table 3.** Amount of TPC, TFC, TTC and TAC in *A. officinalis* leaves extract

TPC (mg GAE/gm of dry extract)	TFC (mg QE/gm dry extract)	TTC (mg GAE/gm of dry extract)	TAC (mg AAE/gm dry extract)
216.21±0.0028	176.09±0.002121	46.35±0.002	126.20±0.63

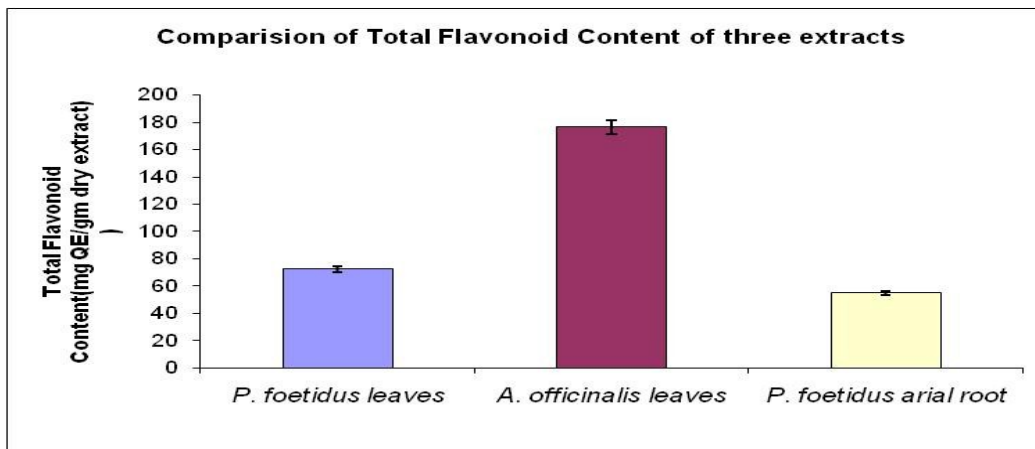
**Figure 1.** The calibration curve of Gallic acid to determine total phenolic content



**Figure 2:** Comparison of total phenolic content of *P. foetidus* leaves, *P. foetidus* aerial root & *A. officinalis* leaves extracts



**Figure 3:** The calibration curve of Quercetin to determine total flavonoids content



**Figure 4:** Comparison of total flavonoid content of *P. foetidus* leaves, *P. foetidus* aerial root & *A. officinalis* leaves extracts



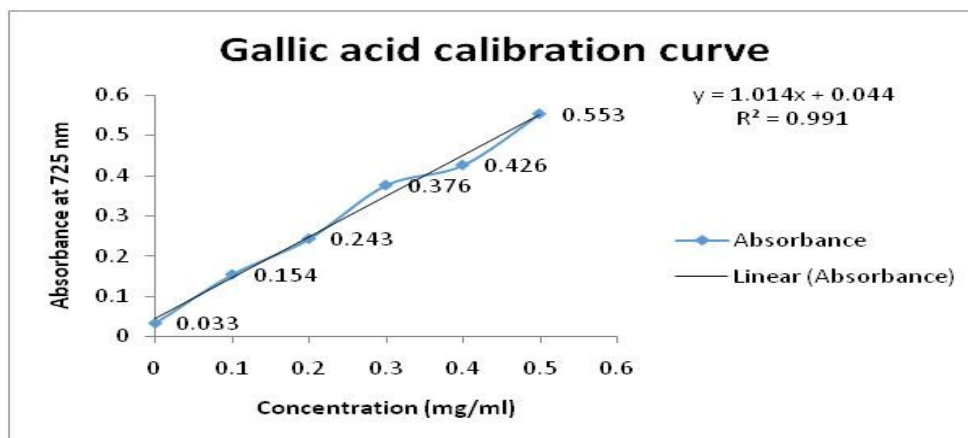


Figure 5: The calibration curve of Gallic acid to determine total tannin content

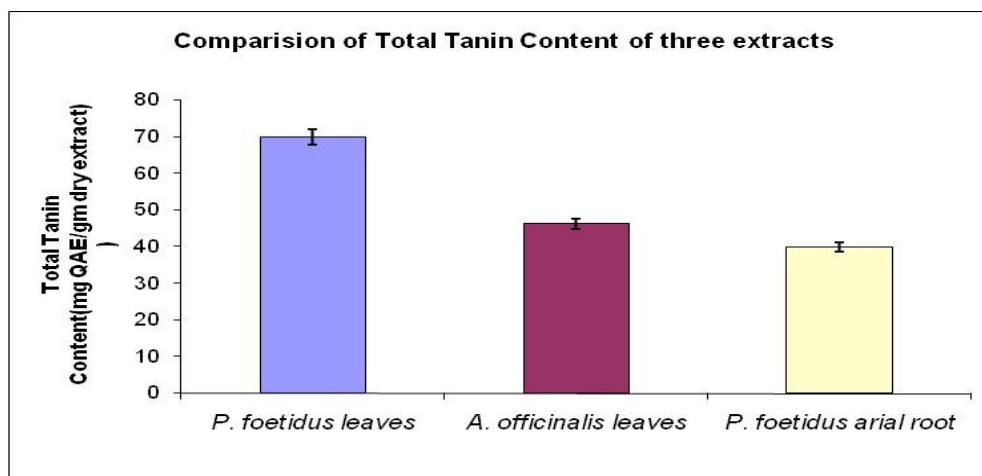
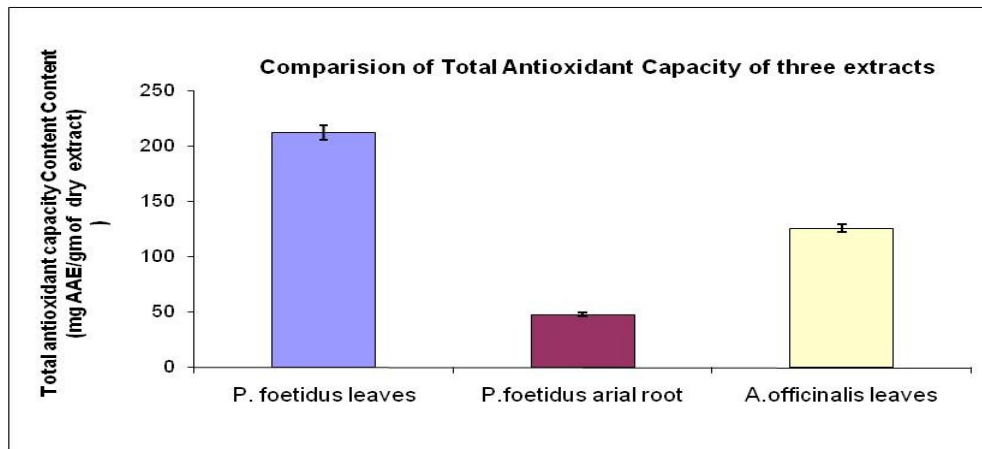


Figure 6: Comparison of total tannin content of *P. foetidus* leaves, *P. foetidus* aerial root & *A. officinalis* leaves extracts.



**Figure 7:** Comparison of total antioxidant capacity of *P. foetidus* leaves, *P. foetidus* aerial root & *A. officinalis* leaves extracts.