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# PHYTOCHEMICAL SCREENING AND PHARMACOLOGICAL STUDIES OF METHANOL EXTRACT OF POLYALTHIA SUBEROSA ROXB.

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## Abstract

The aim of the study was to find out the phytochemical evaluation, antioxidant, cytotoxic and thrombolytic activity from the leaves of the methanolic extract of the plant of *Polyalthia suberosa* Roxb. Phytochemical analysis of *Polyalthia suberosa* Roxb displayed the presence of alkaloids, saponins and tannin types of compounds. The anti-oxidative effect was evaluated using DPPH free radical scavenging activity method. The methanolic extract of *Polyalthia suberosa* Roxb. leaves is found to have antioxidant activity that will be the IC50 value of standard ascorbic acid for DPPH was 1.85 µg/ml and methanol extract of *Polyalthia suberosa* Roxb was 8.48 µg /ml. The methanol crude extract of *Polyalthia suberosa* shows moderate cytotoxic activity against the brine shrimp nauplii. The LC50 value of methanol extract was 0.3119 mg/mL compared with the LC50 value of standard (0.0171 mg/mL). Addition of 100 µl SK, a positive control (30,000 IU), to the clots and subsequent incubation for 90 minutes at 37 °C, showed 88.49% lysis of clot. On the other hand, distilled water was treated as negative control which exhibited a negligible percentage of lysis of clot 10.44%. The results indicated that the extract has moderate antioxidant, cytotoxic and thrombolytic activities.

**Keywords:** *Phytochemical, Antioxidant, Polyathia suberosa, Thrombolytic*

**Introduction**

Plant is an essential source of medicine which keeps a vital figure in world health [1]. Medicinal plants may be defined as those plants which are usually used in treating as well as preventing specific diseases. The WHO (World Health Organization) calculated that more than 75% peoples are widely used herbal drugs for their regular primary healthcare needs [2- 4]. Polyalthia suberosa Roxb. belongs to the family of Annonaceae is an evergreen shrub or small tree growing up to 5 metres tall. The plant has minor edible and medicinal uses. Fruits are numerous, ovoid or globose , 4 to 5 mm long, purple, fleshy and edible. The leaves are 12–22 cm long, pinnate, with 5–11 leaflets, the girth is 12–34 m wide. The flowers are produced in panicles 6–13 cm long containing a few to numerous flowers. Plants of the Polyalthia genus are said to help with a number of health conditions, including: Bladder infections, Cold, Diabetes, Hepatitis B, Kidney disorders, Ulcers, Urinary tract infections. In addition, plants of the Polyalthia genus are said to alleviate pain, improve liver health, and promote wound-healing [5-11].

Plants are a promising source of beneficial compounds for human health and also for drug discovery and development [12]. Mainly bioactive plant metabolites are responsible for the therapeutic properties of the medicinal plants [13]. Phenolic compounds mainly flavonoids and phenolic acids, have several biological properties including antioxidant, antibacterial, anticancer activities [14]. The antioxidant capacity of plant phenolics depends on their concentration [15], number and position of hydroxyl group. These types of compounds are responsible for neutralization of harmful free radicals. Due to overproduction of free radicals and lack of antioxidants a condition known as oxidative stress is developed. Free radical induced oxidative damage has long been thought to be the most important cause of many chronic and degenerative diseases such as diabetes, stroke, cancer, arteriosclerosis, and cardiovascular diseases [16]. These hazardous conditions can be overcome through several plant secondary metabolites including alkaloids, flavonoids, lignins, phenolic compounds and terpenoids [17]. Also phenolic compounds are often considered to play an important role in resistance to many plant pathogens [18]. Thrombolysis, also known as thrombolytic therapy, is a treatment to dissolve excessive clots in blood vessels, improve blood flow, and prevent damaging to tissues and organs. Thrombolysis may be included the injection of clot-busting drugs through an intravenous (IV) line or through a long catheter that delivers drugs directly to the site of the blockage. Thrombolysis is applied as an emergency treatment to dissolve blood clots that form in arteries feeding the heart and brain -- the main cause of heart attacks and ischemic strokes -- and in the arteries of the lungs (acute pulmonary embolism).Thrombolysis is also used to treat blood clots. If a blood clot is calculated to be life threatening, thrombolysis may be an option if induced as soon as possible -- ideally within one to two hours -- after the onset of prefix of a heart attack, stroke, or pulmonary embolism [19].

Cytotoxicity is the quality of being toxic to cells. Examples of toxic agents are an immune cell or some types of venom, e.g. from the puff adder (*Bitis arietans*) or brown recluse spider (*Loxosceles reclusa*). It is based on the ability to kill laboratory cultured Artemianauplii brine shrimp. The assay is considered a useful tool for preliminary assessment of toxicity, and it has been used for the detection of fungal toxins, plant extract toxicity, heavy metals, cyanobacteria toxins pesticides, and cytotoxicity testing of dental materials. The brine shrimp lethality bioassay can be used as a convenient monitor for screening and fractionation in the discovery of new bioactive natural products. Natural product extracts, fractions or pure compounds can be tested for their bio activity by this method. Apoptosis is characterized by well-defined cytological and molecular events including a change in the refractive index of the cell, cytoplasmic shrinkage, nuclear condensation and cleavage of DNA into regularly sized fragments. Cells in culture that are undergoing apoptosis eventually undergo secondary necrosis. They will shut down metabolism, lose membrane integrity and lyse [20-24].

Chemotherapy as a treatment of cancer often relies on the ability of cytotoxic agents to kill or damage cells which are reproducing; this preferentially targets rapidly dividing cancer cells[25,26].

To the best of our knowledge, very few pharmacological studies have been reported so far on the Polyalthia suberosa Roxb. As a part of the continuation of our research on bioactivity screening of Bangladeshi medicinal plants, present study was carried out to assess antioxidant, thrombolytic and cytotoxic activities of leaves extract of Polyalthia suberosa Roxb. inorder to scientifically evaluate the claimed biological activities [27].

**Methods and Materials**

**Collection of Plant materials:**

*Polyalthia suberosa* locally known as kauaduli in Bangladesh (kaua thuti). Polyalthia suberosa is an evergreen shrub or small tree growing up to 5 metres tall. The plant has minor edible and medicinal uses.

The whole plant was collected from National Botanical Garden, Dhaka, Bangladesh. The plant was identified and authenticated by Bangladesh National Herbarium.

**Drying and grinding**

The collected plant parts (Leaves) was separated from undesirable materials or plants or plant parts. They were dried in the sun for one week after cutting into small pieces. The plant parts were ground into coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

**Preparation of Methanol extraction:**

About 560 gm of powdered sample was taken in a clean, flat-bottomed glass container and soaked in 900 mL of 90% methanol. The container with its contents was sealed and kept for a period of 10 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by apiece of clean, white cotton material. Then it was filtered through whatman filter paper.

**Chemicals and Reagents:**

DPPH (2,2-diphenyl-1-picryldydrazyl), Folin-Ciocalteu re- agent, Galli acid, Quercetin, Ascorbic acid and other chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA). Methanol, Distilled water from BDH Chemicals Ltd and Vincristine sulfate from Beacon Pharmaceuticals Ltd. Bangladesh.

**Phytochemical screening:**

Different phytochemical groups such as alkaloids, glycosides, flavonoids, tannins, gums, saponins, steroids were identified by characteristic colour change using standard chemical tests. Molisch Test and Fehling's Test were used for carbohydrate existence. Biurets's Test was used for Proteins detection. Flavonoid Test was used for detection of flavonoids. Alkaloids were detected using the Dragendroff's, Mayer's and Hager's test. For identification of tannin potassium dichromate test, ferric chloride, and lead acetate tests were followed. Keller- Kiliani tests were performed to identify glycosides. Frothing Test for saponins existence, Sulphuric acid test was performed for the detection of steroid. Molisch test was performed for detecting the existence of gum in the samples [28].

**Total flavonoid contents**

Total flavonoid content was measured by the aluminium chloride colorimetric assay. The reaction mixture consists of 1 mL of extract and 4 mL of distilled water was taken in a 10 mL volumetric flask. To the flask, 0.30 mL of 5 % sodium nitrite was treated and after 5 minutes, 0.3 mL of 10 % aluminium chloride was mixed. After 5 minutes, 2 mL of 1M Sodium hydroxide was treated and diluted to 10 mL with distilled water. A set of reference standard solutions of quercetin (20, 40, 60, 80 and 100 µg/mL) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with an UV/Visible spectrophotometer. The total flavonoid content was expressed as mg of QE/g of extract [29-32].

**Total phenolic contents**

The concentration of phenolics in plant extracts was determined using spectrophotometric method. Folin-Ciocalteu assay method was used for the determination of the total phenol content. The reaction mixture consists of 1 ml of extract and 9 mL of distilled water was taken in a volumetric flask (25

mL). One millilitre of Folin-Ciocalteu phenol reagent was treated to the mixture and shaken well. After 5 minutes, 10 ml of 7 % Sodium carbonate ($Na_2CO_3$) solution was treated to the mixture. The volume was made up to 25 mL. A set of standard solutions of gallic acid (20, 40, 40, 60, 80 and 100 µg/mL) were prepared in the same manner as described earlier. Incubated for 90 min at room temperature and the absorbance for test and standard solutions were determined against the reagent blank at 550 nm with an Ultraviolet (UV) Visible spectrophotometer. Total phenol content was expressed as mg of GAE/gm of extract [33-34].

**Total tannin content**

The tannins were determined by Folin - Ciocalteu method. About 0.1 mL of the sample extract was added to a volumetric flask (10 mL) containing 7.5 mL of distilled water and 0.5 mL of Folin-Ciocalteuphenol reagent, 1 ml of 35 % $Na_2CO_3$ solution and dilute to 10 mL with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/mL) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of GAE /g of extract [35-38].

**DPPH radical scavenging assay**

The radical scavenging activity ofthe extract was quantitatively estimated on the basis of its ability to scavenge the free radical 2,2-diphenyl-1-picryl hydrazyl (DPPH). At first stock solution (1024 µg/mL) of the samples was prepared. From that solution different concentrations (512–1 µg/mL) of sample were prepared. In 1 mL of each concentration, 3 mL of 0.1 mM alcoholic DPPH solution was added. After 30 min of incubation in dark at room temperature, absorbance was taken at 517 nm. Ascorbic acid was used as standard. The percentage of DPPH free radical scavenging activity of each extract and standard were calculated as:

DPPH radical–scavenging activity (1%)

¼ ½A0 –A=A0 x 100

Where, A 0 is the absorbance of the control solution containing all reagents except plant extracts, A is the absorbance of DPPH solution containing plant extract. Finally, the concentration of sample required to scavenge 50% DPPH free radical (IC50) was calculated from the plot of inhibition (%) against the concentration of the extract[39].

**Reducig Power assay**

To determine the reducing power of the extract, fromthe stock solution different concentrations (0.1-1 mg/mL) of extract was prepared. In 1 mL of sample solution 0.2 M phosphate buffer (2.5 mL; pH 6.6) and 10 g/L potassium ferricyanide (2.5 mL) were added. The mixturewas incubated at 50°C for 20 min. After cooling at room temperature, 100 g/L trichloroacetic acid (2.5 mL) was added. The mixture was centrifuged at 3000 rpm. For10 min. In the supernatant (2.5 mL), distilled water (2.5 mL) and 1 g/L ferric chloride (0.50 mL) were added.After 10mins, absorbance of the mixture was measuredat 700 nm. Butylated Hydroxy Toluene (BHT) was used as standard [40].

**Thrombolytic activity:**

Preparation of extract dose: Extract Concentration, Stock solution = 100mg/10ml. Standard: Streptokinase 1500000 IU/5ml, Dose: 30000 IU in 100µl

Procedure: In vitro clot lysis activity of the leaves was carried out according to the method with minor modifications. With ethical considerations, and aseptic precaution, 5 ml of venous blood was drawn from healthy volunteers (n = 3) having no history of smoking, taking lipid lowering drugs, oral contraceptive or anticoagulant therapy and transferred to different pre weighed sterile micro-centrifuge tube (1 ml/tube). The micro-centrifuged tubes were subjected to incubation at 37°C for 45 min. After the formation of clot, serum was completely removed from the tubes (carried out without disturbing the clot formed) and each tube having clot was again weighed to determine the weight of the clot (clot weight = weight of clot containing tube – weight of tube alone). To each

micro-centrifuge tube containing pre-weighed clot, 100 µl solution of different extracts, concentration 1 mg/mL, were added accordingly. As a positive control, 100 µl of streptokinase and asn a negative non thrombolytic control, 100 µl of sterilized distilled water were separately added to the control tubes numbered. Then all the tubes were incubated again at 37°C for 90 min and observed for clot lysis. After incubation, the obtained fluid was removed from the tubes and they were again weighed to observe the difference in weight after clot disruption. At last, difference obtained in weight was calculated and the result was expressed as percentage of clot lysis following the underneath equation.

**% of clot lysis = (wt. of lysis clot /initial clot wt.)×100** [41]

**Cytotoxic activity:**

Cytotoxic activity of extract was carried out according to the Meyer method. Artemia salina leach (brine shrimp eggs) was used as the test organism. It was hatched in simulated sea water. Two days were allowed to hatch the shrimp and to be matured as nauplii. Constant oxygen supply was carried out through the hatching time. Different concentration of extract (200, 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781 µg/mL) was prepared using dimethyl sulfoxide (DMSO) in sea water. A set of seven test tubes were used where 10 shrimps were taken and a solution of different concentration was applied on it. At last, the final volume was adjusted with saline water and kept for 24 h. Vincristine sulfate was used as standard. The lethal concentration LC50 of the test samples after 24 h was obtained by a plot of percentage of the shrimps killed against the sample concentration [42].

**Statistical analysis**

One-way ANOVA followed by Dunnett's test were performed and the results were considered statistically significant when $p < 0.05$. The assays were carried out in triplicate and the results are expressed as mean values ± standard deviations.

**Results and Discussion**

Results of the phytochemical screening of the methanol extract of leaves of *Polyalthia suberosa* [table 1]

+ = Indicates the presence of the tested group, – = Indicates the absence of the tested group.

In the phytochemical screening both extracts revealed the presence of some of the pharmacologically active phytochemicals. After completing wide range of chemical test for the identification of major classes of therapeutically important compounds, alkaloid, Glycoside, tannins, steroids, flavonoids, Saponin protein. Biochemical screening results showed that tannins, alkaloids, glycosides, steroids and flavonoids are present but saponins are absent. The following table 1 will give us an idea about phytochemicals.

In case of antioxidant activity, we put concentration along X axis and % inhibition along Y axis $y = 9.538\ln(x) + 0.963$, if y =50 then find out the value of x. That will be the IC50 value of standard ascorbic acid for DPPH was 1.85µg/ml and methanol extract of *Polyalthia suberosa* was 8.48µg /ml where $y = 12.13\ln(x)+3.955$ [Figure1].

The total phenolic contents in the examined plant extracts using the Folin- Ciocalteu's reagent is expressed in terms of gallic acid equivalent (the standard curve equation: $y = 1.75x - 0.312$, $R^2 = 0.751$ ). The values obtained for the concentration of total phenols are expressed as mg of GAE/g of extract. The total phenolic contents in the examined extracts ranged from 1.25 mg GAE/g. [Figure2]

The concentration of flavonoids in various plant extracts of Polyalthia suberosa was determined using spectrophotometric method with aluminum chloride. The content of flavonoids was expressed in terms of quercetin equivalent (the standard curve equation: $y = 1.204x - 0.048$, $R^2 = 0.966$) mg of QE/g of extract. Methanol extracts of plant contains the flavonoid content as 3.1 mg QE/g. [Figure3]

The tannins contents was examined in plant extracts using the Folin-Ciocalteu's reagent is expressed in terms of quercetin equivalent (the standard curve equation: $y = 4.54x - 0.292$, $R^2 = 0.913$. The values obtained for the concentration of tannin contents are expressed as mg of QE/g of extract. The concentration of tannins was measured 2.895mg QE/g[Figure4].

Reducing power of the extract was found to be concentration dependent. The maximum

absorbance of 0.140 was observed at the highest conc. of the extract, i.e., 1mg/ml. BHT, used as the positive control showed maximum absorbance of 1.256 at the same concentration[Figure5].

In case of thrombolytic test, Addition of 100 µl SK, a positive control (30,000 IU), to the clots and subsequent incubation for 90 minutes at 37 °C, showed 88.49% lysis of clot. On the other hand, distilled water was treated as negative control which exhibited a negligible percentage of lysis of clot 10.44%. The mean difference of in percentage of clot lysis between positive and negative control was found to be statistically significant. In this study *Polyalthia suberosa* displayed highest thrombolytic activity 27.27%. [Table 2].

The mortality rate of the brine shrimp was found to be increased with the increasing of concentration of the extract and plotting of Concentration versus Response percentage put on the Ldp Line software produced an approximate linear correlation between them. The concentration at which 50% mortality(LC50) of brine shrimp nauplii caused by the test extract were calculated from the graph by extrapolation and was found LC50 blow in table.

The brine shrimp lethality is a simple, rapid and convenient method for identifying biological activity having cytotoxicity in the crude extract. The methanol crude extract of Polyalthia suberosa shows moderate activity against the brine shrimp nauplii. The LC50 value of methanol extract was 0.3119 mg/mL compared with the LC50 value of standard (0.0171 mg/mL). Therefore, the response obtained in this assay suggests that the extract may contain cytotoxic compounds. However, this can't be confirmed without further higher and specific tests. So, further investigations are needed to get more information about the activities of the plant [Table 3].

## Conclusion

For thousands of years there have been plants in India used for medicinal purpose, while many studies have attempted to prove scientifically on these medicinal plants. Polyalthia suberosa is one of the important medicinal plants among them. Hence, further studies are required to find out more pharmacological activities of this plant.

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## References

1.Sandberg F, Corrigan D. Natural remedies: their origins and uses. CRC Press; 2001.

2. Schulz V, Hänsel R, Tyler VE. Rational phytotherapy: a physician's guide to herbal medicine. Psychology Press; 2001.

3. Bodeker G, Ong CK. WHO global atlas of traditional, complementary and alternative medicine. World Health Organization; 2005.

4. Sagnia B, Fedeli D, Casetti R, Montesano C, Falcioni G, Colizzi V. Antioxidant and anti-inflammatory activities of extracts from Cassia alata, Eleusine indica, Eremomastax speciosa, Carica papaya and Polyscias fulva medicinal plants collected in Cameroon. PloS one. 2014;9(8):e103999.

5. Kirtikar K R, Basu B D. Indian Medicinal Plants. Vol.1, International book distributors: Dehradun; India 1995.

6. Sharmila B G, Kumar G, Rajasekara P M. Cholesterol-Lowering Activity of the Aqueous Fruit Extract of Trichosanthes dioica Roxb (L.) in Normal and Streptozotocin Diabetic Rats, J.Clin.diag.res,2007; 1:561-569

7. USDA. Natural Resources conservation systems. Classification. Available from https://plants.usda.gov/java/ClassificationServlet?source=display&classid=TRDI7

8. S.K. Sen, L.M. Behera Ethnomedicinal plants used by the tribals of Bargarh district to cure diarrhoea and dysentery Ind. J. Tradit. Knowl. 2008;7; 425-428

9. Health benefits times. Com. Health benefits of Pointed Gourd/ Parwal. Available from https://www.healthbenefitstimes.com/health-benefits-of-pointed-gourd-parwal/

10. Pointed Gourd Health Benefits, Usage - Ayurveda Details. Available from http://easyayurveda.com/2013/02/08/pointed-gourd-health-benefits-usage-ayurveda-details/

11. McLauglin JL, Chang C J, Smith DL: Bench top" bioassay for the discovery of bioactive natural products: an update. In Studies in Natural Products Chemistry. Edited by AU Rahman. Elsevier; 1991:383-409.

12. Kouloura E, Genta-Jouve G, Pergola C, Krauth V, Litaudon M, Benaki D, Wertz O, Michel S, Mikros E, Skaltsounis L, Halabalaki M. Dereplication and metabolomics strategies for the discovery of bioactive natural products: the Acronychia example. Planta Medica. 2014;80(16):SL3.

13. Silva O, Duarte A, Cabrita J, Pimentel M, Diniz A. Antimicrobial activity of Guinea-Bissau traditional remedies. J Ethnopharmacol. 1996; 50(1):55–59.

14. Zhang Q, Cui H. Simultaneous determination of quercetin, kaempferol, and isorhamnetin in phytopharmaceuticals of Hippophae rhamnoides L. by high-performance liquid chromatography with chemiluminescence detection. J Sep Sci 2005;28(11):1171– 1178.

15. Dobre I, Dâdei G, Patrascu L, Elisei AM, Segal R. The antioxidant activity of selected Romanian honeys. AUDJG. Food Technol. 2010;34: 67–73

16. Barriada-Bernal LG, Almaraz-Abarca N, Delgado-Alvarado EA, Gallardo-Velázquez T, Ávila-Reyes JA, Torrres-Morán MI, González-Elizondo MS, Herrera-Arrieta Y (2014) Flavonoid composition and antioxidant capacity of the edible flowers of Agave durangensis (Agavaceae). CyTA J Food. 2014; 12:105–114.

17. Saleem M, Nazir M, Shaiq A, Hussain H, Lee YS, Riaz N, Jabbar A. Antimicrobial natural products:anupdateonfuture antibiotic drug candidates. Nat Prod Rep. 2009; 27(2):238–254.

18. Lyon GD, Heilbronn J, Forrest RS, Johnston DJ. The biochemical basis of resistance of potato to soft rot bacteria. Neth J Plant Pathol. 1992; 98(2):127–133.

19. Dobak III JD, Lasheras JC, inventors; Innercool Therapies Inc, assignee. Method for low temperature thrombolysis and low temperature thrombolytic agent with selective organ temperature control. United States patent US 6,478,811. 2002

20. Riss TL, Moravec RA; Moravec. "Use of multiple assay endpoints to investigate the effects of incubation time, dose of toxin, and plating density in cell-based cytotoxicity assays". Assay Drug Dev Technol. 2004;2 (1):51–62

21. Decker T, Lohmann-Matthes ML. A quick and simple method for the quantitation of lactate dehydrogenase release in measurements of cellular cytotoxicity and tumor necrosis factor (TNF) activity. Journal of immunological methods. 1988;115(1):61-9.

22. Niles AL, Moravec RA, Hesselberth PE, Scurria MA, Daily WJ, Riss TL. A homogeneous assay to measure live and dead cells in the same sample by detecting different protease markers. Analytical biochemistry. 2007;366(2):197-206.

23. Fan F, Wood KV; Wood. "Bioluminescent assays for high-throughput screening". Assay Drug Dev Technol. 2007;5 (1):127–36

24. Dearden, J. C. "In silico prediction of drug toxicity". Journal of computer-aided molecular design. 2003;17 (2–4): 119–27.

25. Ramin Zibaseresht, Photoactivated Cytotoxins, University of Canterbury, 2006.

26. "Chemotherapy Principles" (PDF). American Cancer Society. Retrieved t 2014.

27. Zilani MN, Sultana NA, Bakshi MK, Shampa IJ, Sumi SJ, Islam O. Bioactivities of leaf and root extract of Ceriscoids turgida (Roxb.). Oriental Pharmacy and Experimental Medicine. 2018:1-7.

28. Ghani A. Practical phytochemistry. Parash Publishers, Dhaka, 2005;pp 8–20

29. S Kaviarasan, GH Naik, R Gangabhagirathi, CV Anuradha, KI Priyadarsini. In vitro studies on antiradical and antioxidant activities of fenugreek (Trigonellafoenumgraecum) seeds. Food Chem. 2007; 103: 31–37.

30. Hanane El Hajaji, NadyaLachkar, KatimAlaoui, YahyaCherrah, Abdellah Farah, AbdesslamEnnabili. Antioxidant Properties and

Total Phenolic Content of Three Varieties of Carob Tree Leaves from Morocco. Rec Nat Prod. 2010; 4(4): 193- 204.

31. Xu BJ, Chang SK. Total phenolic content and antioxidant properties of eclipse black beans (Phaseolus vulgaris L.) as affected by processing methods. J Food Sci. 2008; 73(2): H19-27.

32. Lee Wei Har, IntanSafinar Ismail. Antioxidant activity, total phenolics and total flavonoids of Syzygiumpolyanthum (Wight) Walp leaves. Int J Med Arom Plants. 2012; 2(2): 219-228.

33. Ghasemzadeh A, Jaafar HZ, Rahmat A. Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (Zingiber officinale Roscoe). Molecules. 2010;15(6):4324-33.

34. Stankovic MS. Total phenolic content, flavonoid concentration and antioxidant activity of Marrubium peregrinum L. extracts. Kragujevac J Sci. 2011;33(2011):63-72.

35. D Marinova, F Ribarova, M Atanassova. Total Phenolics and Total Flavonoids in Bulgarian Fruits and Vegetables. J University ChemTechnol Metallurgy. 2005; 40 (3): 255-260.

36. Rajeev Singh, Pawan Kumar Verma, Gagandeep Singh. Total phenolic, flavonoids and tannin contents in different extracts of Artemisia absinthium. J Intercult Ethnopharmacol. 2012; 1 (2): 101-104.

37. AfifyAel-M, El-Beltagi HS, El-Salam SM, Omran AA. Biochemical changes in phenols, flavonoids, tannins, vitamin E, β-carotene and antioxidant activity during soaking of three white sorghum varieties. Asian Pac J Trop Biomed. 2012; 2(3): 203-9.

38. Miean K.H, Mohamed S. Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. J Agric Food Chem. 2001; 49 (6): 3106-12.

39. Anisuzzman M, Zilani MN, Khushi SS, Asaduzzman M. Antioxidant, antibacterial potential and HPLC analysis of Dioscorea alata bulb. Indonesian Journal of Pharmacy. 2016;27(1):9.

40. Zilani MN, Sultana T, Rahman SA, Anisuzzman M, Islam MA, Shilpi JA, Hossain MG. Chemical composition and pharmacological activities of Pisum sativum. BMC complementary and alternative medicine. 2017;17(1):171.

41. Bhowmick R, Sarwar MS, RahmanDewan SM, Das A, Das B, NasirUddin MM, Islam MS, Islam MS. In vivo analgesic, antipyretic, and anti-inflammatory potential in Swiss albino mice and in vitro thrombolytic activity of hydroalcoholic extract from Litsea glutinosa leaves. Biological research. 2014;47(1):56.

42. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DJ, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Planta medica.1982;45(05):31-4

**Table 1.** -Phytochemical test results of barks extract of *Polyalthia suberosa*

| Tested groups | Methanol extract of *Polyalthia suberosa* leaves |
|---|---|
| Tannins | + |
| Alkaloids | + |
| Glycosides | + |
| Saponins | - |
| Steroids | + |
| Flavonoids | + |

**Table 2:** Thrombolytic activity (in terms of % clot lysis) of *Polyalthia suberosa*

| Sample | Blank tube weight (gm) | 1st clot + tube weight (gm) | 1st clot weight (gm) | 2nd clot + tube weight (gm) | 2nd clot weight (gm) | % of lysis |
|---|---|---|---|---|---|---|
| Standard (Streptokinase) | 0.838 | 1.663 | .825 | .9335 | .0955 | 88.49% |
| Control (Distil water) | 0.824 | 1.456 | 0.632 | 1.390 | 0.066 | 10.44% |
| *Polyalthia suberosa* methanolic Leaves extract | 0.840 | 1.471 | 0.88 | 1.350 | 0.64 | 27.27%. |

**Table.3:-** Cytotoxic study of *Polyalthia suberosa* against brine shrimp nauplii.

| Serial no. | Control | | Standard | | Methanol extract | |
|---|---|---|---|---|---|---|
| | No. of Alive | No. of Death | No. of Alive | No. of Death | No. of Alive | No. of Death |
| 01. | 09 | 01 | 00 | 10 | 00 | 10 |
| 02. | 08 | 02 | 00 | 10 | 01 | 9 |
| 03. | 08 | 02 | 00 | 10 | 02 | 8 |
| 04. | 09 | 01 | 00 | 10 | 03 | 07 |
| 05. | 08 | 02 | 02 | 08 | 04 | 06 |
| 06. | 08 | 02 | 03 | 07 | 05 | 05 |

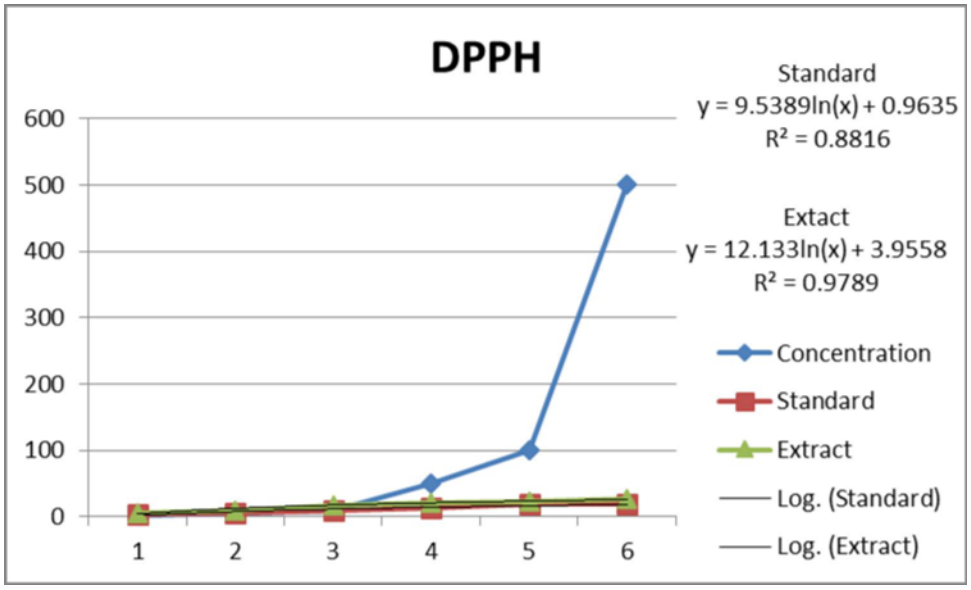


**Figure-1:** Curve of DPPH scavenging Antioxidant activity

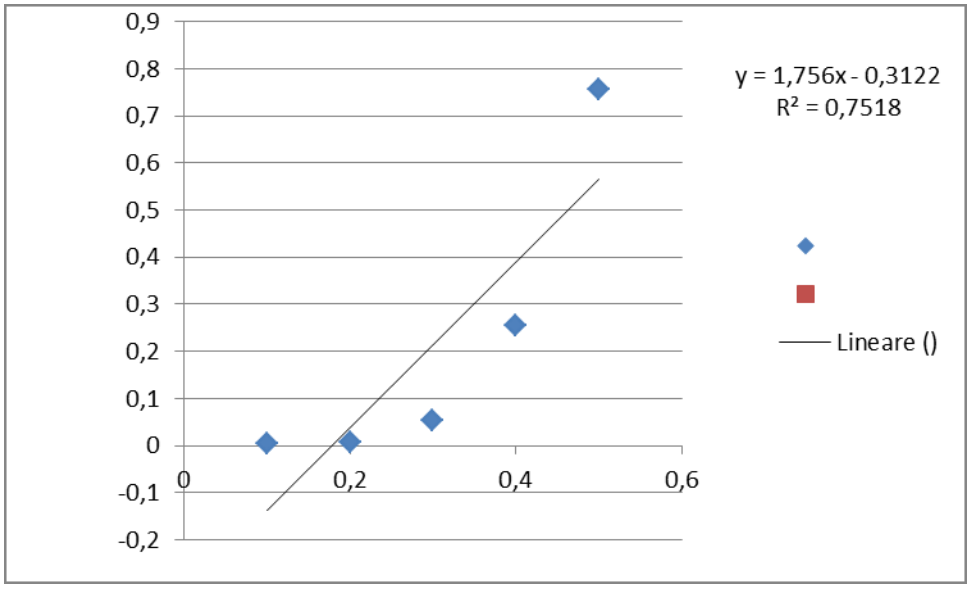


**Figure-2:** Total Phenolic Contents

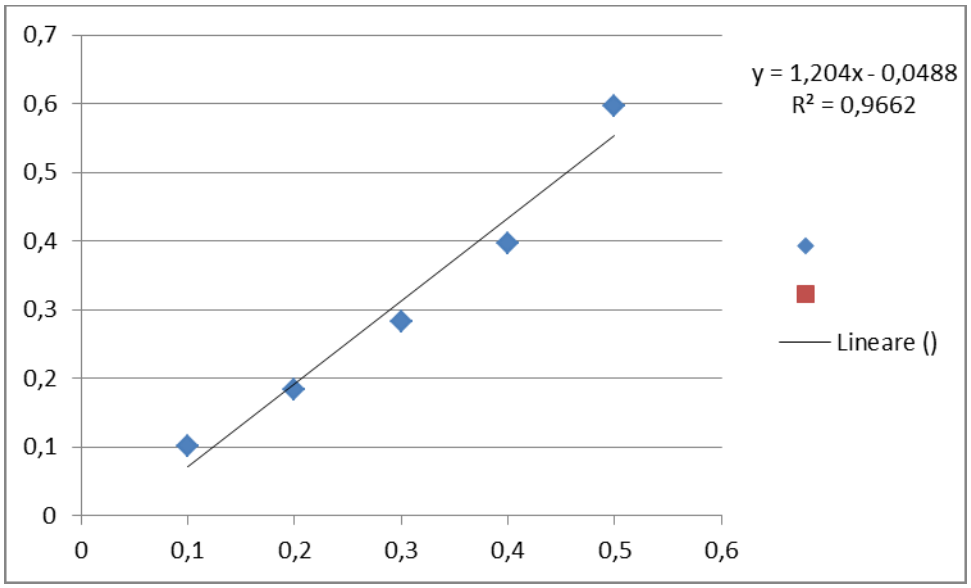


**Figure-3.** Total Flavonoid Contents

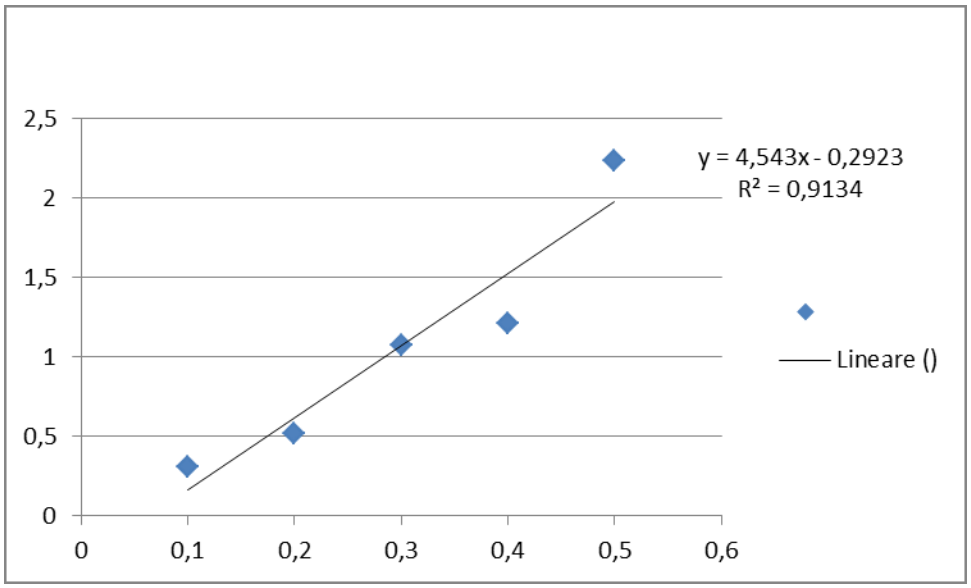


**Figure- 4:** Total Tannin Contents

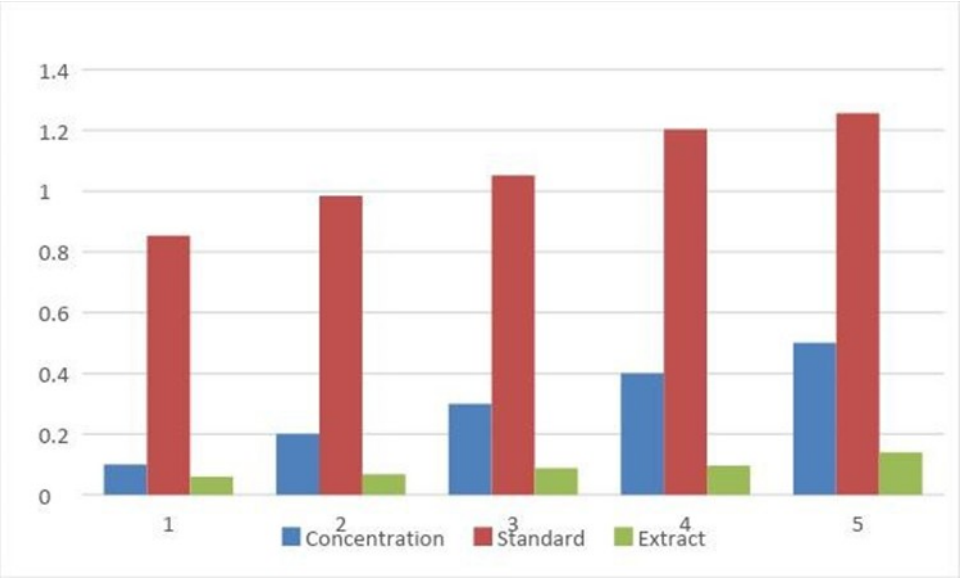


**Figure- 5:** Reducing Power assay