



Original Research

In vitro phytochemical analysis, antimicrobial and antioxidant activity of *Mallotus Philippinensis*

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Abstract

Background: *Mallotus Philippinensis* belongs to the family Euphorbiaceae was broadly utilized in traditional medicines especially in the tropical areas. Mostly distributed in the Himalaya from Kashmir East wards besides tropical India. The plant contains high content of steroids, diterpenoids, triterpenoids, flavonoids, phenols, proteins, saponins, alkaloids and carbohydrates. The previous studies demonstrated that plant exerted significant pharmacological effects like anti-helminthics, antifilarial, antiparasitic, antiulcer, antifungal, antibacterial and immuno-regulatory properties, and as an aphrodisiac. Still antimicrobial activities from different solvent extracts of this plant needed to explore. **Objective:** The goal of the present investigation was to evaluate the phytochemical constituents, antibacterial, antifungal and antioxidant activity from the extract of plant *Mallotus Philippinensis*. **Method:** The ethanolic crude extract of plant was extracted by using maceration method. The resulting extracts were used to examine the antibacterial activity by disc diffusion method against some clinical strains (*Bacillus*, *E. coli*, *S. aureus*, *Salmonella*, *Epidermidis*, *E. faecalis*, *Pseudomonas*) and some ATCC strains (*Bacillus*, *E. coli*, *S. aureus*, *K. pneumonia*, *Pseudomonas*) and Phytoconstituents was also investigated for the presence or absence of various metabolites like proteins, carbohydrates, alkaloids, steroids flavonoids and saponin test. The DPPH method was used to determine the antioxidant activity. **Results:** The ethanol extract exhibit positive results for the inhibition of *Bacillus*, *E. coli*, *S. aureus*, *Salmonella*, *Epidermidis*, *E. faecalis*, *Pseudomonas* and also exhibit positive results for ATCC strains *Bacillus*, *E. coli*, *S. aureus*, *K. pneumonia*, *Pseudomonas*. The extract showed the presence of proteins, steroids, carbohydrates, alkaloids, flavonoids and saponin in the phytochemical screening. DPPH free radical scavenging activity assay disclosed the remarkable antioxidant activity of different parts of *Mallotus Philippinensis* ethanolic crude extracts. **Conclusion:** The findings of present project indicated that ethanolic extracts of *Mallotus Philippinensis* are sensitive against test bacteria. They also show antioxidant activity, and they contain rich number of phytoconstituents. In current study only crude extract was investigated for different curative effects against certain standard microbial strain. In future these extracts were considered after purification for development of various therapeutic agents.

Introduction: Generally, *Mallotus philippinensis* L. termed as Kameela or Kamala and a huge wooded multiuse remedial tree. It included in the family of Euphorbiaceae consisting of basils, ferns and herbs. It possessed height about 10-12 meter showing in figure 1, and broadly dispersed completely in the Himalaya from Kashmir east wards and moist areas of India up to 5000 feet. The several parts of the Kamala were used for the curing of problems of skin, bronchitis, diarrhea, urinogenital infection, cancer, diabetes, jaundice, antifungal tape worm, eye-disease, malaria, etc [1, 2].

It was stated that fruits indicating in figure 1(d-e) of kameela had used from the very long time in Chinese traditional Medicine systems, Arabic, and ayurvedic in india as anti-helminthics, antifungal, antiulcer, antifilarial, antiparasitic, as an aphrodisiac, antibacterial and immunoregulatory properties [3]. The saturated constituents contained myristic and palmitic acids [4]. Other active constituents were flavonoids, steroids, diterpenoids, coumarins, cardenolides, triterpenoids, isocoumarins, and mainly phenols like rottlerin, bergenin and isorottlerin [5, 6].

Kameela had played a vital role for maintaining of human health as source of medicinal plant subsequently from the past [7]. *Mallotus philippinensis* small much evergreen tree having branches as showing in figure 1. The plant was found useful in cathartic, antioxidant, astringent, anthelmintic, blood purifier, styptic [8, 9]. It also effective in bile trouble, boils, eruptions of skin weeping eczema, pimples and frackles [10, 11]. The fruits contained rottlerin, flavanone chromene 2' 2' 6' trihydroxy - 2' 2' dimethyl pyrano 6" 5" 4", 5 - chalcone, 5-7 dihydroxy-6 methyl-8-prenyl flavone [12, 13], cinnamoylchromene. The current study yielded useful information which could be used for the successful treatment of lot of ailments.

The goal of the current analysis is indeed to evaluate the Efficacy or ability to scavenge radicals of 50% ethanol extract from Mallotes philippinensis. Human erythrocytes were tested in vitro to determine the phenolic and flavonoid contents, which may be responsible for the antihemolytic effect. As exogenous antioxidant systems from the diet, phenolic compounds can protect against oxidative damage caused by ROS and free radicals. A number of ROS scavenging activities were tested in the extract, including superoxide radical, hydroxyl radical, nitric oxide, and lipid peroxidation. ROS were included in mechanisms related to metabolic disorders and endothelial dysfunction. In order to counteract these damages, natural antioxidant compounds rich in phenols and flavonoids can be helpful. However, the precise molecular mechanisms of phenols, flavonoids, and other antioxidants are still a matter of considerable research. In an analysis of antioxidant activity carried out using standard DPPH and ABTS methods, plant extracts were found to be extremely potent.

Classification:

| | |
|----------|------------------------|
| Kingdom- | Plantae |
| Order- | Malpighiales |
| Family- | Euphorbiaceae |
| Genus- | <i>Mallotus</i> |
| Species- | <i>M. Philippensis</i> |

Botanical Explanation: Seeds of kamala are sub globose and of black color and 4 mm transversely. Trees of kameela possessed variety of size. They might be of small

and also medium, and also found as monoecious tree, with a diameter of 50 cm for the bole, up to 25 m tall and but mostly much less in number. Branchlets are reddish brown glandular. Slash turning deep red. Leaves are generally grave or acute at peak, clearly 3-nerved, hairy and rose-colored glandular beneath, the size of petioles exhibits length 1-4 cm and reddish brown in color. It had simple and alternative leave, comparatively fibrous, oval to lanceolate and from base it showed rounded form with 2 glands. The fruit is a depressed globose with 3 seeds; the capsule has three lobes; there are 5 glandular granules surrounding the seeds; the male flowers are in axillary and terminal positions, 2-10 cm long with paniculate spikes, with several stamens on each flower. Flower segments contain spines or slender racemes, filamentous bracts, 3 celled ovary, and 3 papillose stigmas on the female flowers. [140].

Biology: Fruits mature in July-August and Flowers mature from March - April in this genus, *Mallotus philippinensis* (*M. philippinensis*) having more flowered nectaries that attracted by ants as showing in figure 1(d,e).

Ecology: Kameela tree present in perennial forest, particularly in secondary forest, and occasionally prevailing in the underbrush. Kameela tree resists extensive shade; it unaffected by to drought and frost-hardy. *M. Philippinensis* had universal natural, 7mm, and 10mm; puberulous; with plentiful orange and scattered in western Himalayas, to southern China India, Sri Lanka, and throughout Malesia to Australia. At times it possessed pleasant but commonly assorted with more species present in both open bushes and forests.

Biophysical Limits: Mostly plant grow in broad kinds of varieties of soil, included limestone, acid, infertile soils, and rocky land. Kameela tree generally developed at an elevation of 0-1600-meter mean rainfall ranges from 800-2000 mm per year, with a mean temperature of 16-28°C.

Traditional Uses: Ayurveda showed that, all parts of plant like glands and hairs from the capsules or fruits might be utilized as carminative, heating, cleansing, detergent and alexiteric and are advantageous in cure of bronchitis, abdominal infections, and spleen expansion, and it could be somewhat beneficial for expelling tapeworms if taken with milk and curd, leaves are unpleasant, and starter. Kameela, in short utilized as an oral contraceptive [15].

Common Adulterants: *M. philippinensis* glandular hair powder is commonly mixed with ferric oxide, brick dust, and ferruginous sand. Annato dye (*Bixaorellana* Linn), *Ficusbenghalensis* (fruit powder), *Carthamustinctorius* (flower powder), used as additive or ancillary of kameela [16].

Chemical Components: Kameela oil had reported to contained around 60% of kamlolenic acid as merely chief element with fewer quantities of common linoleic, and oleic acid.

Pharmacological action of Kamala: Antileukemic activity, antioxidant hepatoprotective, Antibacterial, antiviral, anti-diabetic, immunomodulatory and antifungal anticancer activity [17, 18].

Compound Unani Formulations: Kameela was active constituents of some compound formulations such as Roghan Kameela, Zimad Jarb [19, 20].

Materials and Methods: Plant material: Genome mapping patent plant collected from Bhimber Azad Kashmir. From

March to April during fruiting season the plant was collected and was proved and recognized.

Sample Extraction: The cold maceration method was used for the extraction of plant material. Fresh plant was collected and separated its all parts stem, leaf, bark and fruit. Proximately 60g of each part was added to 350 ml of ethanol in blue cap bottle respectively and for 4 days in gloominess at 37, then filtered each part separately, after 4 days with the help of funnel in flask. Ethanolic extract settle down in flask after filtering, then placed each part of ethanolic extract in 4 petri dishes respectively. Placed petri dishes at room temperature for 2 days, finally after 2 days ethanol evaporated, and crowd extract settle down in petri plates. Took Eppendorf and weigh it in weighing balance. With the help of surgical blade crowd extract were put in Eppendorf from petri dishes, then weigh Eppendorf's again. All sample extraction processes done by following previously reported data [20].

Qualitative phytochemical estimation: The crude ethanolic extract of genome mapping patent plant analyzed by standard methods and fundamental study was finalized by chemical test followed by [23].

Test for carbohydrates: Molisch test: 2 ml of Molisch's reagent mixed with ethanolic crude extract of plant and the solution was chattered accordingly. Then concentrated H_2SO_4 2ml let flow delicately alongside of test tube. Violet color appeared at the interphase confirmed the carbohydrates. Benedict's test: 2ml of Benedict's reagent mixed with ethanolic crude extract of each plant and heated, A Reddish-brown precipitate appeared that indicated carbohydrates present.

Test for Saponins: 5ml of distilled water in test tube when mixed with ethanol crude extract and quiver actively. Saponins confirmed when stable foam was formed.

Test for Terpenoids: 2ml of chloroform mixed with ethanolic crude extract and dehydrated, and then after adding and heating concentrated H_2SO_4 2ml for 2 min a grayish color showed that terpenoids presence.

Detection of Flavonoids: Alkaline reagent test: When 2ml of 2% solution of NaOH mixed with ethanol crude extract an intense yellow color appeared by adding some droplets of acid (diluted) they become colorless which directed towards flavonoids.

Test for Phenols: 2ml of 2% solution of $FeCl_3$ mixed with ethanolic crude extract. A blue-black or green color appeared that confirmed phenol.

Test for Tannins: 2ml of 2% solution of $FeCl_3$ mixed with ethanolic crude extract. A blue-black or green color appeared that confirmed the tannins.

Test for Steroids: When 2ml of chloroform and concentrated H_2SO_4 mixed with ethanolic crude extract added alongside. In lower chloroform layer red colored showed that steroid present.

Test for Alkaloids: When 2ml of 1% HCL mixed with ethanolic crude extract and heated evenly and then added Mayer's reagent to the mixture. Turbidity of resulting precipitates were considered as proof of alkaloids.

Test for proteins: Ninhydrin test: 2ml of 0.2% solution of Ninhydrin with ethanolic crude extract when boiled, then violet color formed which showed that proteins are present.

Test for Glycosidase: Keller Killani test: When 2ml of glacial acetic acid containing 1 or 2 drops of 2% solution of $FeCl_3$ mixed with ethanolic crude extract. Then took

another test tube contained 2ml of concentrated H_2SO_4 and poured mixture into it. Brown rings formed that was evidence of presence of cardiac glycosidase.

Antimicrobial Activity: Bacterial isolates: Some clinical strains were used including *Bacillus*, *E-coli*, *S-aureus*, *S-epidermidis*, *E. faecalis*, *Pseudomonas*, *salmonella* and some ATCC culture were used including *Bacillus*, *E-coli*, *S aureus*, *k-pneumoniae*, *Pseudomonas* and identified on the basis of morphology and biochemical characteristics by using selective and differential media. The cultural were preserved in glycerol and a working stock was prepared by using methodology [23].

Some strains were used seven were clinical and five were ATCC namely *Escherichia coli*, *Pseudomonas Aeruginosa*, *S-aureus*, *S. epidermidis*, *E. faecalis*, *Salmonella*, *Bacillus* and ATCC 61738, *Pseudomonas* ATCC, *K-pneumonia* ATCC, *E. coli* ATCC, *S-aureus* ATCC, *B. cereus*, ATCC and 3 fungal strains namely *Aspergillus niger*, *Aspergillus flavous*, *C- albicans* were used through the analysis. Clinical strain preserved at department of Microbiology, institute of medical science UOL Lahore. From American type culture collection (ATCC), 5 cultures were obtained.

Preparation of sample extract for microbiology assay: To obtain stock solution, approximately 1mg of extract was mixed in 1ml DMSO respectively. Now we prepared working solution. Firstly, weighed Molar Hinton agar.

To prepare Molar Hinton agar for 12 petri dishes, weighed the molar Hinton agar 9.1g on weight balance. Then poured it in flask and added 240ml water in it. Shake it well and covered it with aluminum foil and let it place in autoclave for 1 hour, warped the petri dishes with paper and placed in hot air oven to sterilize. Molar Hinton agar made by driving 2ml of molten media to the sterilized petri dishes along both sides having flame. For 10 min plates having media permitted to get hard. Let it incubate for 24 hours. After incubation of 24 hour, observed that no contamination is present. To prepare nutrient broth 3.2g put it in round bottom flask and added 250ml of distilled water in it. Shake it vigorously then with the help of glass pipette poured 10ml of nutrient broth in each test tube, let it place for 1 hour to autoclave. Then labelled test tubes by names of strains. Strains were present in glycerol stock. Inoculated strains (present in glycerol stock) in test tubes according to the names of strains in between flame. Let it incubate for 24 hours. Then prepared discs from Whatman filter paper, placed them in petri plates. Took ear picks and placed them in petri plates. Sterilized the (disc and ear pick) in hot air oven at 150°C for 1 hour. After 24 hour it was observed that growth of strains formed in test tubes. Labelled petri plates by their names of strains. Some of them are ATCC and some are clinical. Working area must be sterilized and placed flame on both sides. Bacteria that were grown in test tube. With the help of ear picks, pick up the loop full culture from test tubes and swap it on petri plates having same name of test tube and petri plates. Named the petri dishes in three columns as leaf, stem, and bark. Pick up the discs with the help of forceps and dip in the Eppendorf's in which solution of extract is present that was prepared in DMSO. Placed three discs of leaf extract on petri plates (molar Hinton agar) one by one. Same for leaf, fruit, and bark each. Process is repeated for all stains. Incubated all petri plates in incubator for 24 hours at 37 °C.

There were inhibition zones around the discs at the end of incubation, and measured them with transparent scale in mm. Triplicate was carried for accurate results

Determination of minimum inhibitory concentration

MIC: According to approved methods agar dilution test performed. Inoculate prepared by swing growth in Muller Hinton broth to turbidity of 0.5 McFarland standard from overnight culture. Final inoculates had 10^4 organism. Incubated dishes whole night at 35°C, the lowermost concentration of ethanolic extract showed the minimum growth shown as MIC.

Antifungal Activity: For antifungal activity SDA (sabouraud dextrose agar) was prepared by weighing in weight balance of 16.4g and added 250ml distilled water, wrapped it with aluminum foil. Placed in autoclave for half an hour. Wrapped petri plates and placed them into hot air oven to sterilize. After autoclave let it cool down for a while and poured in petri plates with flame on both sides. Leave it to solidify and incubate it for 24 hours. At the end of incubation, no contamination was present. Took normal saline and poured in test tube. Placed test tube to autoclave for 1 hour. There were 4 petri plates having *Aspergillus Niger*, *Aspergillus flavous*, *C-albicans*. Pick up loop full culture from petri plate and inoculate in test tubes between flames around them. Incubated for 24 hours. After incubation fungal growth was grown in test tube.

Working area must be sterilized and placed flame on both sides. Bacteria that were grown in test tube. With the help of ear picks, pick up the loop full culture from test tubes and swap it on petri plates having same name of test tube and petri plates. Named the petri dishes in three columns as leaf, stem, and bark. Pick up the discs with the help of forceps and dip in the Eppendorf in which solution of extract is present that was prepared in DMSO. Placed three discs of leaf extract on petri plates (Molar Hinton agar) one by one. Same for leaf, fruit and bark each. Process is repeated for all stains. Incubated all petri plates in incubator for 24 hours at 37 °C. There were inhibition zones around the discs, at the end of incubation and with the help of transparent ruler they were measured in mm. In triplicate, entire process carried out. Followed by (17).

Antioxidant Activity: DPPH radical scavenging activity was determined by adopting the reported method using different parts of the plant ethanolic extract *Mallotus philippinensis* (18).

To prepare 0.1Mm DPPH solution, 0.02g extract and sample were dissolved in 20ml ethanol to prepare 1000µg/ml stock solution of extract and sample.

Different dilutions of 125µg/ml, 250µg/ml, 500µg/ml and 1000µg/ml were prepared from the stock solution. In 3 mL of DPPH, the stock solution and dilution prepared above were first mixed and shaken vigorously. The mixture was allowed to stand at room temperature for 60 minutes. After the specified time, the absorbance of the above mixture was measured in a spectrophotometer at 517nm using ethanol as a blank. A positive control (l-ascorbic acid) was also run. Percentage scavenging (%) = $\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$

Results: Phytochemical Analysis: Phytochemical tests had been carried out for screening of ethanolic extract of *Mallotus philippinensis* plant. Table no 1 indicating the presence of different phytochemicals present in different

parts of plant confirmed by different biochemical tests, which may be leading to antibacterial activity.

Anti- bacterial assay: To check the antibacterial activity extracts were analyzed against different strains of bacteria like *S. aureus*, *E. coli*, *Pseudomonas Aeruginosa*, *Epidermidis*, *E. faecalis*, *Salmonella*, *Bacillus* and ATCC 61738, *Pseudomonas ATCC*, *K-pneumonia ATCC*, *E.coli ATCC*, *S-aureus ATCC*, *B. cereus*, ATCC and 3 fungal strains namely *Aspergillus niger*, *Aspergillus flavous*, *C-albicans*. Zone of inhibition was measured in mm diameter of clearance. The results obtained against different bacterial strains were presented in following Table no .2.

Anti- fungal assay: To check the anti-fungal activity extracts were analyzed against three fungal strains namely *Aspergillus niger*, *Aspergillus flavous*, *C-lbicans* respectively. Zone of inhibition was measured in mm diameter of clearance. The results obtained against different bacterial strains were presented in following Table no .3.

Antioxidant activity: Estimation of antioxidant activity of *Mallotus philippinensis* plant extract in ethanolic solvent by comparing the standard ascorbic acid with the help of statically analysis. The obtained significant results were presented in Table no. 4. The graphical representation of these results indicated a clear comparison between different parts as showing in figure 2. Data showed that ethanolic stem and bark extract of *Mallotus philippinensis* exhibits remarkable activity closely to the ascorbic acid.

Statistical analysis: Mean values were calculated from triplicate analysis collected results and presented in tables. Microsoft excel sheets were used for drawing of bar charts and graphs.

Discussion: A phytochemical screening of methanolic extracts of *Mallotus philippinensis* indicated that *alkaloids*, *phenolic groups*, *steroids*, *flavonoids*, *phenols*, *saponins*, *steroids*, *sugars*, *tannins* and *triterpenes* are present in it [21] and current work performed on ethanolic extract of *Mallotus philippinensis* also showed the presences of many phytochemical compounds such as alkaloids, phenolic groups, steroids, flavonoids, phenols, saponins, steroids, sugars, tannins and terpenoids. Anti-bacterial efficiency examined on the basis of secondary metabolites. The extract was used against bacteria. It showed resistance against bacterial growth [22] According to recent work on antibacterial efficiency, the extract of kameela showed the similar results, it has remarkable effect against the bacteria, it shows visible zone of inhibition.

In-vitro test results of anti-microbial activity showed the liability test by on no account antifungal beside grams positive and negative bacteria. In contrast to wholly tests organisms against standard drug methanol extract demonstrated significant level of inhibition. This is indicative of the existence of few classes in extract to comparable mechanism of action in contrast to standard drug utilized. Lowest activity demonstrated by residual portion in contrast to *Bacillus subtilis* (8±0.68 mm and highest activity demonstrated by crude extract in contrary to *Pseudomonas aeruginosa* (24±0.52 mm) and). Though, this might propose that plant extracts showing diameters of zones of inhibition > 10mm measured active, current work showed that wide-ranging anti-bacterial activities of extract of the plant had secondary metabolites present in extracts. Tannins had broadly utilized for the medication

of various diseases like strains, damages and superficial injuries. Plant extract of *Mallotus philippinensis* might be responsible for pharmacologic activities. Ethanolic extract was tested against the organism namely *E. coli*, *S. aureus*, leaf, *Bacillus* (stem 26mm, leaf 18mm, bark 17mm), *Pseudomonas* (stem 26mm, leaf 22mm, bark 20mm), *E. faecalis*, *Salmonella*, *Epidermidis*. A widely used medicinal plant, *Mallotus philippinensis*, contains molecules that have strong antioxidant properties. Previous studies have demonstrated their antibacterial and wound-healing capabilities. They showed magnificent results on DPPH method [23, 24].

Conclusion: The current analysis performed to determine the antioxidant and antibacterial activity or phytochemical screening of plant *Mallotus philippinensis* in ethanol extracts. It was concluded from the results that the plant has significance importance as an antibacterial drug and have potential to kill above mentioned isolates. DPPH radical scavenging activity assay disclosed the remarkable antioxidant activity of *Mallotus philippinensis* ethanolic crude extract.

Limitations: In current study only ethanolic extract of different plant parts were investigated for therapeutic effects against certain standard pathogenic microbial strains. Moreover, crude extract utilized for different in-vitro analysis.

Future prospects: In future purified compound from different plant parts could be applied for drug development mechanism. Different solvent should be utilized for extraction in order to get maximum potential phytoconstituents from this plant. Moreover, experimentation against wide pathogens should be performed.

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Table 1. Phytochemical constituent of MP leaf, Bark and stem Ethanolic extract

| Phytoconstituents | Plant Extract (Ethanol) | | |
|----------------------|-------------------------|--------|------|
| | Stem | Leaves | Bark |
| Alkaloids | - | + | + |
| Benedict's (red sug) | + | + | + |
| Saponins | + | - | + |
| Terpenoids | + | + | + |
| Flavonoids | - | - | - |
| Molish's | + | + | + |
| Tannins | + | + | + |
| Phenol | + | + | + |
| Steroids | + | + | + |
| Glycosidase | + | + | + |
| Ninhydrin | - | - | - |

Table 2. Zone of inhibition table of seven bacterial strains at 20 mg/mL concentration

| Strains | Plant Extract | | |
|---------------------------|---------------|-------------|-----------|
| | Stem (mm) | Leaves (mm) | Bark (mm) |
| Bacillus (clinical) | 26 | 18 | 17 |
| Bacillus (ATCC) | 17 | 21 | 17 |
| E-coli (clinical) | 17 | 15 | 17 |
| E-coli (ATCC) | 20 | 17 | 15 |
| S-aureus (clinical) | 25 | 20 | 25 |
| S-aureus (ATCC) | 15 | 14 | 13 |
| S. Epidermidis (clinical) | 15 | 15 | 17 |
| K-pneumonia (ATCC) | 19 | 20 | 17 |
| E. facillus (clinical) | 15 | 14 | 15 |
| Pseudomonas (clinical) | 26 | 22 | 20 |
| Pseudomonas (ATCC) | 16 | 20 | 13 |
| Salmonella (clinical) | 16 | 15 | 15 |

Table 3. Zone of inhibition table of fungal strain at 20 mg/ml concentration

| Strains | Plants Extract | | |
|--------------------|----------------|------------|-----------|
| | Leaves (mm) | Stems (mm) | Bark (mm) |
| Aspergillus niger | 15 | 15 | 14 |
| Aspergillus flavus | 12 | 13 | 15 |
| C-albicans | 15 | 20 | 13 |



(a) Mature plant



(b) Leaf



(c) Initial inflorescence of seed setting



(d) Mature fruit twig



(e) Mature fruit with seed

Fig. 1. *Mallotus philippinensis*

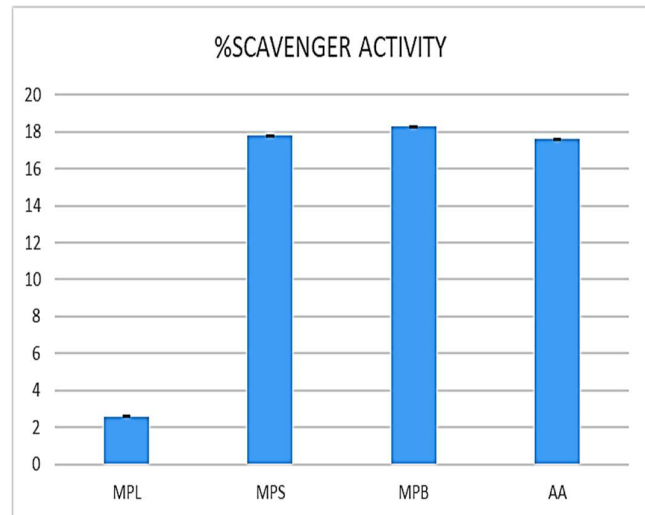


Fig. 2. A line graph indication comparison between antioxidant activities of different parts of plant