# Qualitative and quantitative photochemical analysis of amla (*Emblica officinalis*) and henna (*Lawsonia inermis*)

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**Abstract:** The present research work was undertaken with this aim to inspect the two medicinally important plants henna (*Lawsonia inermis*) of the family lythraceae and amla (*Emblica officinalis*) of family phyllanthaceae for their phytochemical analysis using ethanol and methanol reagents. The determinations under ethanol and methanol dilution of *L. inermis* for qualitative screening of tannins were positive, phlobatannins were positive, saponins were positive and in flavonids test results were also found positive. While the determinations under ethanol and methanol dilution of *E. officinalis* for qualitative screening of tannins test gave positive results, its phlobatannins gave positive results but less; saponins also gave positive results and flavonoids gave negative results. And then the determinations under ethanol and methanol dilution of *L. inermis* for quantitative screening of total carbohydrates were 6.547 and 6.276 µg/ml, reducing sugars 0.035 and 0.259 µg/ml, total proteins 0.245 and 0.420 µg/ml, phenolic compounds were 27.1191 and 35.2316 µg/ml, total flavonoids contents 0.154 and 0.261 µg/ml, tannins contents 0.664 and 1.292 µg/ml, and antioxidant activity were 2.411 and 3.218 µg/ml respectively.

**Keywords:** henna; amla; qualitative; quantitative; medicinal plants.

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## 1 Introduction

Medicinal plants have a vital role in the well-being of people because of their therapeutic efficacy and healing potential (Cordell, 2000). The importance of medicinal plants is indispensable to human development (Petrovska, 2012). Various plant parts such as roots, leaves, seeds, and bark are useful due to their therapeutic, tonic, purgative, or other health-promoting purposes (Foster and Duke, 2000). According to the World Health Organization (WHO, 2019), about 80% of the world's population uses herbal medicine.

Traditional medicine is an inexpensive alternative source of medicine at the level of primary healthcare in parts of the world that lack modern health facilities (Aziz et al., 2018). The medicinal plant and its parts are a rich source of active principal compounds that help us to alleviate human suffering and certainly have paved the way in the formulation of numerous life-saving drugs in use today (Rupani and Chavez, 2018).

Medicinal plants are proved to be an important biological source of drugs and are used as folk medicines, traditional medicines, nutraceuticals and as pharmaceutical intermediates (Hao, 2019). The phytochemicals of medicinal plants origin are the probable source to cause some definite physiological actions on the human body (Ncube et al., 2008). The data showed that about 3.3 billion people in developing countries use therapeutic drugs, and therefore conventional medicine is buffered by medicinal plants. The use of medicinal plants dates long back in our yesteryears; in fact, as old as human civilisation (Petrovska, 2012).

Medicinal plants continue to inspire new pharmaceutical substances, which provide a crucial chemical structure for the development of new antimicrobials and phytomedicine (Abukakar et al., 2008). Secondary plant metabolites (phytochemicals) have been studied extensively as a source of drug substances in recent years with previously unknown pharmacological activities (Krishnaraju et al., 2005). Different plant molecules, including vitamins, terpenoids, phenol acids, lignins, stilbanes, tannins, flavonoids, chinones, coumarins, alkaloids, amines, betalans, and other antioxidant-rich metabolites (Zhang et al., 2001).

Medicinal plants of natural habitats gains more scientific attention as their systematic medicinal properties are long been in practice also their uses are described in ancient Unani Egyptians and Chinese writings, that the Unani Hakims, Indian Vaid, European and Mediterranean cultures including Romans, Egyptians, Iranians, Africans, Americans, and all others have clued us about their herbal therapies that had now been employed systematically (Tapsell et al., 2006). Ancient China residents are commonly accepted for use of herbal medicines; the first paper on the systemic use of the traditional medicinal plant awareness in the eastern Han Dynamas in Shennong (AD-220AD) is the Bencao Jing (Shennong's Herbal Classic) (Sun et al., 2014).

Traditional medicinal plants are significant to protect the lives of people of different ethnic minorities, particularly from remote and less developed regions (Liu et al., 2009; Kidane et al., 2018). Plant-based chemicals and active ingredients are the main sources of medicine, their concentration and types treat the various disorders, most are inhibitory antioxidants, oxidising certain chemical molecules help transfer electrons or hydrogen from a substance to an oxidant, creating the freely available radicals between antioxidant molecules some of them are flavonoids, vitamin E, vitamin C and phenolic compounds (Nekeety et al., 2011).

The study is comprised of a phytochemical analysis of two medicinal plants which included henna (*Lawsonia inermis*) and amla (*Emblica officinalis*). The extracts of these plants were determined for their chemical compositions. Keltoum and Mohamed (2017) mentioned that the flowers yielded more essential oil than the leaves and the seeds respectively. Native Plants (2016) is the document on the subject of native plants, as henna being a source of organic dye; is nature's gift for fashion to be used in the eastern and western societies.

Amla is a magnificent and precious natural gift to man; due to his high health and nutritional value, he has great potential (Thind, 2019). Vitamin C, amino acids, and

minerals are the richest source of Anon (2017). It comprises different chemical components such as tannins, alkaloids, and phenols (Zhang et al., 2004). Sheikh (1993) illustrated that amla fruit is hard when it is ripe, it weighs approximately between 60 and 70 grams. Amla ash contains 2.5 ppm, chromium, zinc, and 3 ppm of copper (Anon, 2017). The present study was carried out for beneficial aspects of human beings and better understanding of phytochemical analysis of amla (*Emblica officinalis*) and henna (*Lawsonia inermis*)

## 2 Material and methods

The plant's parts used for different remedies by local people were bought from the grocer's shop.

**Table 1** Showing the botanical names, common names, family names, and parts used

| *Botanical name* | *Common name* | *Family name* | *Part used* |
|---|---|---|---|
| *Lawsonia inermis* | Henna | Lythraceae | Leaves |
| *Emblica officinalis* | Amla | Euphorbiaceae | Seed |

The research was carried out in the laboratories of the Institute of Biotechnology and genetic engineering and the hi-tech laboratory resource of the University of Sindh Jamshoro Pakistan was also used.

## 3 Extract formation

The plant material at its first was surface washed to free it from debris and other contaminants, followed by double-distilled waters, and then it was air-dried at room temperature (26°C) and grounded to uniform fine powder with a pestle motor. After that methanol and ethanol extracts were prepared by taking 20 grams of powder of each plant part and they were mixed with 40–45 ml of ethanol and methanol chemicals and solutions are formed. Later these solutions were poured into a centrifuge tube and solutions were centrifuged for 10 minutes, the filtrate of these solutions was obtained, the obtained filtrates were then filtered with the help of filter paper into small bottles, in prepared solution more 35 ml ethanol and methanol were added, and samples were frozen for 48 hours.

### *3.1 Qualitative screening of photochemical*

Qualitative screening of photochemical from two plants *Lawsonia inermis* and *Emblica officinalis* are as under:

1 test for tannins

2 test for phylobatannins

3 test for saponins

4 test for flavonoids.

### *3.2 Procedure for qualitative phytochemical analysis*

Qualitative phytochemical analysis of henna and amla aqueous extracts was performed on the extract using standard methods to classify the components as defined Soni and Sosa (2013) and Nipoku and Obi (2009).

#### *3.2.1 Test for tannins*

A quantity of 1 ml of each test was taken in separate test tubes and then 1 ml of 0.008 M potassium ferric cyanide was applied to each measuring sample. Additional 0.1 N HCL-containing 1 mL of 0.02 M ferric chloride. Tannins are found in research samples when the appearance is a transient greenish to blue colouring (Soni and Sosa 2013).

#### *3.2.2 Test for phytobatannins*

The plant was initially extracted with 2% aqueous hydrochloric acid and was cooked with 2 ml of each plant extract sample. Red precipitation deposition confirms phlobatannin existence (Soni and Sosa, 2013).

#### *3.2.3 Test for saponins*

The saponin chemical compounds extracted from plant samples were prepared; mix each plant sample with 5 ml of distilled water and shake vigorously, then add the sample with a few drops of olive oil, The formation of stable foam was an indication for the presence of saponins (Soni and Sosa 2013).

#### *3.2.4 Test for flavonoids*

1 ml of plant extract material was collected in test tubes in each test sample and applied a 10% lead acetate solution with only a few drops. A yellow appearance florescence precipitates were taken as the positive test for flavanoids (Nipoku and Obi, 2009).

### *3.3 Quantitative screening of phytochemicals*

Quantitative screenings of phytochemicals from two plants henna (*L. inermis*) and amla (*Emblica officinalis*) as under:

1 determination of total carbohydrates

2 determination of reducing sugars

3 determination of total proteins

4 determination of phenolic compound

5 determination of total flavonoid contents

6 determination of tannin contents

7 determination of antioxidants.

### 3.3.1 Determination of total carbohydrates

To determine total carbohydrate contents of the prepared sample by the reporting process phenol-sulphuric acid (Nielsen, 2010). The total 0.5 ml (from each sample of marked extract leaves) of the 0.5 ml test sample was used in marked test tubes. The 2.5 ml sulphuric acid concentrated in each test tube and 80 UL phenol solution in each test tube were loaded. The phenol-sulphuric acid method reported total carbohydrate content (Nielsen, 2010). In testing tubes, the cumulative 0.5 ml of carbohydrates was taken (from any sample of labelled extracts). The 2.5 ml concentrated sulphuric acid and 50 UL of 80% phenol solution were filled for each test tube.

### 3.3.2 Determination of reducing sugars

The reducing sugar contents, of prepared samples as calculated by the recorded process (Miller, 1959). Test samples were taken in test tubes of 1 ml size (from each labelled leaf sample) and 1 ml of freshly prepared 2, 6-DNA reagent was applied to all test tube samples. Test tubes plugged in cotton blended very well and left for five minutes cooling in boiling water, and tubes to cool for a couple of minutes at room temperature. Test tubes plugged with cotton, mixed very well, and all samples were left to be heated in a boiling water bath for five minutes and tubes were taken to be cooled at room temperature for a few minutes. Using the UV visible spectrophotometer against the blank, the sample colour was read at 450 nm. The blank was designed by adding 0.5 ml of 70% methanol to the test sample replacement. Various glucose concentrations of 0.2, 0.4, 0.6, 0.8, 1.0 mg/ml were used to map standard sugar curves. The values obtained were plotted and concentrations of test samples were measured.

### 3.3.3 Determination of total proteins

The method was reported by Lowery et al. (1951) for determining the total protein content of prepared samples is fairly simple. 0.5 ml of the test sample (from each labelled leaf extract) was taken into labelled test tubes and 2.5 ml of alkaline copper reagent was applied to determine the total protein content of plant samples. The solution was then blended well and left to stand for 10 minutes at room temperature to complete the reaction. The solution with a Folin-Ciocalteau reagent of 0.25 ml was applied and the whole sample remained until the bluish colour was seen for 30 minutes. Using a UV-visible spectrophotometer against the blank, the absorption was read at 750 nm. If the sample is checked with all reactions, instead the blank was formed by 0.5 ml of 70% methanol. Various bovine albumin protein strands for the calibration curve have been prepared. Different bovine albumin concentrations (Fluka), 0.1, 2, 0.3, 0.4, 0.5 mg/ml, and the actual research concentration sample were determined from the protein calibration curve for the absorption strand vs. concentration.

### 3.3.4 Determination of phenolic compound

The total phenolic content in henna (*Lawsonia inermis*) and amla (*Emblica officinalis*) extracts from methanol and ethanol was calculated by the Folin-Cocalteus reporting method (Yasoubi et al., 2007). In 1 ml of 10 fold diluted reagent Follin-Ciocalteu, 0.2 ml was applied to the test sample. Added 0.8 ml 7.5% $Na_2CO_3$ (ten-fold was prepared by 1 ml of Follin-Ciocalteu with 9 ml of water). The sample solution was well combined and

all equipment was left for 30 minutes at room temperature. With 0.2 ml 70% methanol, Blank was prepared by replacing testing samples. At 765 nm in UV-visible spectrophotometer, the absorption against the blank was determined.

### *3.3.5 Determination of total flavonoid contents*

The total flavonoid content of henna (*Lawsonia inermis*) and amla (*Emblica officinalis*) aluminium chloride extracts, calculated by the colorimetric aluminium chloride process (Kim et al., 2003). 0.1 ml test sample is applied with 0.3 ml (5%) sodium nitrate (MERCK) and equipment were left at room temperature for five minutes, after 0.3 ml of 10% chloride aluminium (MERCK) had been added, the apparatus had again been left at room temperature for 5 min. Instead of a test sample, the blank was prepared using 0.1 ml of 70% methanol. At 510 nm, the absorption was measured against the blank. The regular absorbance vs. concentration curve was calculated with different quercetin concentrations of 0.2, 0.4, 0.6, 0.8, 1.0 mg/ml, and the true quercetin calibration curve concentration test samples were calculated.

### *3.3.6 Determination of tannin contents*

The tannin content was calculated using Folin and Ciocalteu methods in the methanol and ethanol extract of henna (*Lawsonia inermis*) and amla (Tamilselvi et al., 2012). Added 0.1 ml of the test sample and purified water of 7.5 ml. Added Folin-Ciocalteu (1:1 v/v) 0.5 ml and combined with the following reagent. Then 1 ml of 35% sodium carbonate solution and 10 ml of clean water were diluted. The mixture was well shaken and kept for 30 minutes at room temperature. At 725 nm the absorbance was measured. Instead of the test sample, blank was developed with 70% methanol. Normal absorption vs. concentration curve was determined using a number of gallic acid concentrations as 0.2, 0.4, 0.6, 0.8, 1.0 mg/ml and the real gallic acid calibration curve was calculated with the test levels.

### *3.3.7 Determination of antioxidants*

Their methanol and ethanol extracts were tested for their antioxidant activities by two medically essential plants, henna (*Lawsonia inermis*) and amla (*Emblica officinalis*) (Prieto et al., 1999). A sample of 0.2 ml with 2 ml reagent has been applied (0.6 M sulphuric acid. 28 mM sodium phosphate and 4 mM ammonium molybdate). At 950 C the water bath incubation time was 90 minutes. The test tubes were foiled using aluminium foil and test tube sample samples were placed into a boiling bath (digital constant temperature tank). The samples were then refrigerated at room temperature and the absorption was controlled at 696 nm against blankness. Different levels of α-Tocopherol were used for the normal uptake vs. concentrations of 0.5, 1.0, 1.5, 2.5, 2.0, 2.5mg/ml, and α-Tocopherol (MERCK) calibration curve for the actual concentration of the test sample.

## 4 Results

The laboratory studies were carried out on the phytochemical analysis of henna (*Lawsonia inermis*) and amla (*Emblica officinalis*) using ethanol and methanol reagents with the objectives to do a qualitative analysis of tannins, phlobatannins, saponnins and flavonoids to do quantitative analysis of total carbohydrates, reducing sugars, total proteins, phenolic compounds, total flavonoids contents, tannins contents and antioxidant activity. The results so achieved are presented in graphical illustrations (Table 2), interpreted and discussed as follows.

**Table 2** Qualitative screening of phytochemicals: (methanol and ethanol)

| *Qualitative test* | *Henna (Lawsonia inermis)* | | *Amla (Emblica officinalis)* | |
|---|---|---|---|---|
| | *Reagent* | | *Reagent* | |
| | *Ethanol* | *Methanol* | *Ethanol* | *Methanol* |
| Tannins | Positive (++++) | Positive (++++) | Positive (++++) | Positive (++++) |
| Phlobatannins | Positive but less (++) | Positive but less (++) | Positive but less (++) | Positive but less (++) |
| Saponins | Positive (++++) | Positive (++) | Positive (++++) | Positive (++) |
| Flavonoids | Positive (++++) | Positive (++++) | Negative (-ve) | Negative (-ve) |

### *4.1 Qualitative screening of phytochemicals*

#### *4.1.1 Test for tannins*

The greenish or blue-black colour appeared that indicated (Table 2) the presence of tannin in henna (*Lawsonia inermis*) and amla (*Emblica officinalis*).

#### *4.1.2 Test for phlobatannins*

The red precipitates appeared that indicated (Table 2) the presence of phlobatannins in henna (*Lawsonia inermis*) and amla (*Emblica officinalis*).

#### *4.1.3 Test for saponins*

The stable foam was formed that indicated (Table 2) the presence of saponins in henna (*Lawsonia inermis*) and amla (*Emblica officinalis*).

#### *4.1.4 Test for flavonoids*

The yellow colour precipitates appeared that indicated (Table 2) the presence of flavonoid in henna (*Lawsonia inermis*) and amla (*Emblica officinalis*).

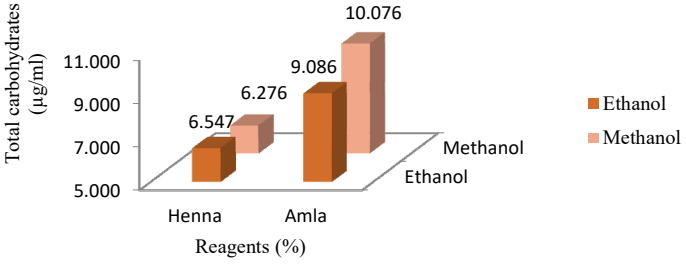
### *4.2 Quantitative screening of phytochemicals*

#### *4.2.1 Determination of carbohydrates*

The carbohydrates in henna (*Lawsonia inermis*) and amla (*Emblica officinalis*) were determined by adopting standard methods under laboratory conditions; while ethanol and methanol reagents were compared for providing significant ($P < 0.05$) results. The results

so achieved are depicted in Figure 1. It was noted that carbohydrates were markedly higher in *Emblica officinalis* extract (10.076 µg/ml) when methanol was used for dilution; while carbohydrates determination in *Emblica officinalis* decreased to 9.086 µg/ml under ethanol extract. In the case of *Lawsonia inermis*, the carbohydrates were 6.547 and 6.276 µg/ml under ethanol and methanol extracts, respectively. It indicated that carbohydrates were markedly higher in *Emblica officinalis* as compared to *Lawsonia inermis* Moreover, methanol proved to be a more effective reagent to produce significant ($P < 0.05$) results for determining carbohydrates in *Emblica officinalis* while in *Lawsonia inermis* ethanol extract showed relative accuracy to determine carbohydrates.

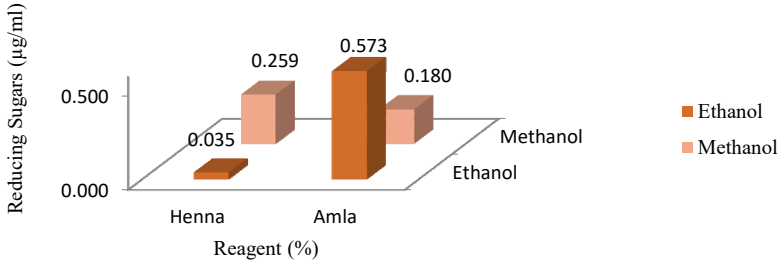
**Figure 1** Determination of total carbohydrates (20%) in henna and amla determined from 20% ethanol and methanol extracts (see online version for colours)




### *4.2.2 Determination of reducing sugars*

Reducing sugars in henna (*Lawsonia inermis*) and amla (*Emblica officinalis*) were analysed by standard methods using ethanol and methanol extracts. The results so achieved are shown in Figure 2. The data indicated that reducing sugars were appreciably higher (0.573 µg/ml) in the ethanol extract of *Emblica officinalis*, while reducing sugars determination decreased to 0.180 µg/ml under the methanol extract of *Emblica officinalis*. In the case of *Lawsonia inermis*, the reducing sugars were 0.259 and 0.035 µg/ml under methanol and ethanol extracts, respectively. Methanol reagent proved to be more effective to provide accurate results in *Lawsonia inermis*; while in *Emblica officinalis* ethanol extract proved to be a more effective reagent to produce accurate results for determining reducing sugars.

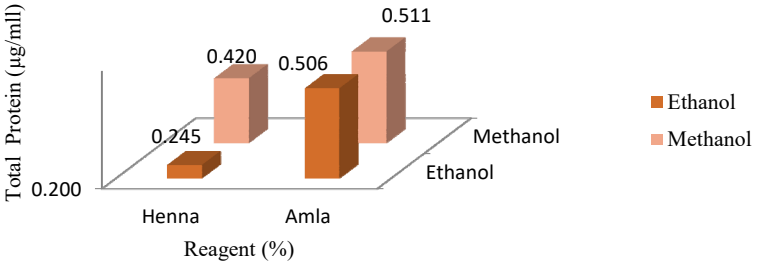
**Figure 2** Total reducing sugar (20%) in henna and amla determined from 20% ethanol and methanol extracts (see online version for colours)



### 4.2.3 Determination total proteins

The total proteins in henna (*Lawsonia inermis*) and amla (*Emblica officinalis*) were determined using ethanol and methanol extracts by standard laboratory procedures. The results illustrated in Figure 3, total proteins were markedly higher 0.511 and 0.506 µg/ml in methanol and ethanol extracts of *Emblica officinalis*. In the case of *Lawsonia inermis*, the total proteins were 0.420 and 0.245 µg/ml under methanol and ethanol extracts, respectively. In plant extracts the determined values for total proteins were higher under methanol diluted extract as compared to ethanol dilution. However, ethanol extract remained ineffective to provide accurate total protein determination in *Lawsonia inermis*; while in *Emblica officinalis* the difference in total proteins determination was negligible under methanol and ethanol extracts. This showed that the methanol reagent was relatively effective to provide significant ($P < 0.05$) results in *Lawsonia inermis* and *Emblica officinalis* as compared to ethanol extract.

**Figure 3** Total proteins (20%) in henna and amla determined from 20% ethanol and methanol extracts (see online version for colours)




### 4.2.4 Determination phenolic compounds

The phenolic compounds in henna (*Lawsonia inermis*) and amla (*Emblica officinalis*) were determined by standard procedures and methods using ethanol and methanol reagents and the results are demonstrated in the following graph (Figure 4). The data show that in *Emblica officinalis* the phenolic compounds were 41.9281 µg/ml and 41.3381µg/ml when ethanol and methanol reagents were used respectively, while in *Lawsonia inermis* samples the phenolic compounds were 27.1191 µg/ml and 35.2316 µg/ml under ethanol and methanol dilutions respectively. In both of the plant extract samples there the phenolic compounds regardless of reagents were higher in *Emblica officinalis* compared to *Lawsonia inermis* samples, while both in *Emblica officinalis* and *Lawsonia inermis* the determined values of phenolic compounds were higher when methanol was used as reagent compared to ethanol reagent. The variation in determining values of phenolic compounds under the influence of ethanol and methanol reagents were exceptionally significant ($P < 0.05$) in the case of *Lawsonia inermis* samples while it was found non-significant ($P > 0.05$) in samples of *Emblica officinalis*.

### 4.2.5 Determination of flavonoids contents

The flavonoids in *Lawsonia inermis* and *Emblica officinalis* plants were tested to determine their flavenoids contents by the use of ethanol and methanol extracts by

standard laboratory protocol. The results so achieved are illustrated in Figure 5. The results showed a higher flavonoid relative value 0.796 and 0.668 µg/ml in methanol and ethanol extracts of *Emblica officinalis*, while in the case of *Lawsonia inermis*, the maximum of flavonoids found were 0.261 and 0.154 µg/ml under methanol and ethanol extracts, respectively. In both the plant extracts the determined values for flavonoids were higher under methanol diluted extract as compared to ethanol dilution. The results confirmed that the methanol reagent was relatively more effective to provide significantly ($P < 0.05$) results in *Lawsonia inermis* and *Emblica officinalis* as compared to ethanol extract for flavonoids determination.

**Figure 4** Phenolic compound (20%) in henna and amla determined from 20% ethanol and methanol extracts (see online version for colours)

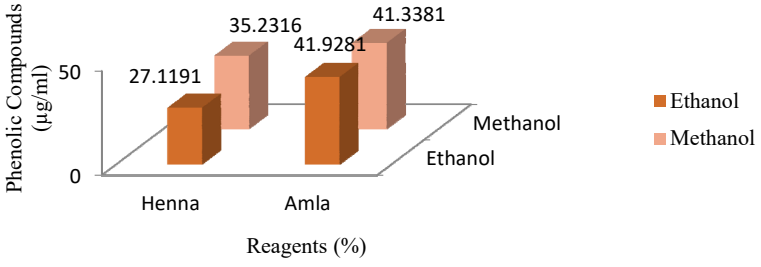
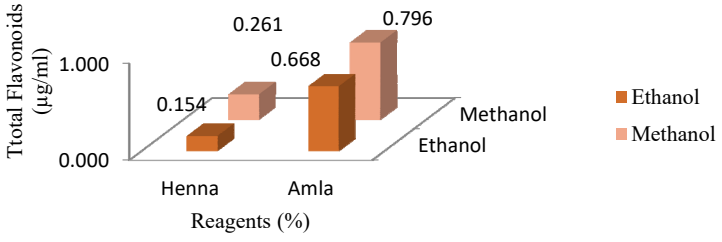


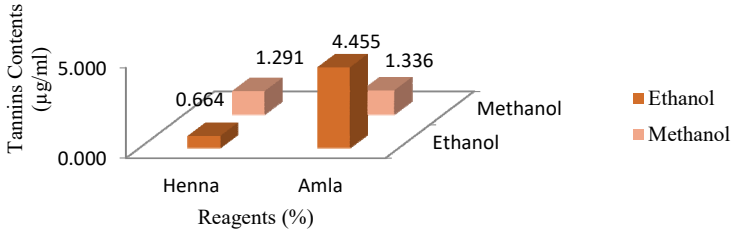

**Figure 5** Flavonoids (20%) in henna and amla determined from 20% ethanol and methanol extracts (see online version for colours)




### *4.2.6 Determination of tannins contents*

The *Lawsonia inermis* (henna) and *Emblica officinalis* (amla) plants were determined for their tannins contents using ethanol and methanol extracts by standard laboratory protocols. The data results of laboratory analysis for tannins are illustrated in Figure 6. It was observed that tannins content were exceptionally higher of 4.455 µg/ml in ethanol extracts of *Emblica officinalis*; which declined to 1.336 µg/ml in *Emblica officinalis* when methanol was used as a reagent. In the case of *Lawsonia inermis*, the tannins were appreciably higher 1.292 µg/ml under methanol and extract, and declined to 0.664 µg/ml in ethanol dilution. For the determination of tannins in *Emblica officinalis* plants ethanol extract was more effective to provide ($P < 0.05$) results; while in *Lawsonia inermis* methanol extract showed ($P < 0.05$) for tannins determination.

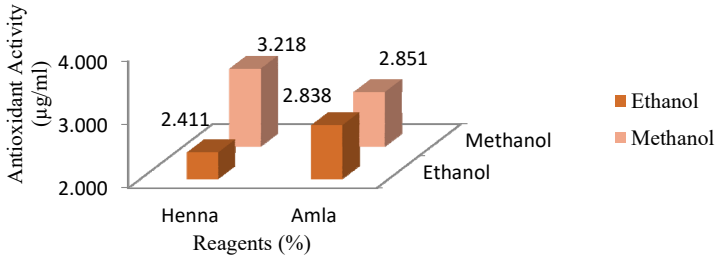
**Figure 6** Determination of tannins contents (20%) in henna and amla determined from 20% ethanol and methanol extracts (see online version for colours)




### *4.2.7 Determination of antioxidant activity*

The antioxidant activity of henna (*Lawsonia inermis*) and amla (*Emblica officinalis*) was analysed using standard procedures and compared by diluting ethanol and methanol reagents. The results so obtained are illustrated in Figure 7. It was observed that antioxidant activity was markedly higher in *Lawsonia inermis* (3.218 µg/ml) when methanol was used for dilution; while antioxidant activity of *Lawsonia inermis* decreased to 2.411 µg/ml when ethanol was used for dilution. In the case of *Emblica officinalis*, the antioxidant activity showed similarity (2.838 and 2.851 µg/ml), when ethanol and methanol were used as reagents, respectively. The antioxidants are inhibitory of oxidation; their oxidation effects via certain chemical reactions cause free radicals, which in turn lead to chain reactions that could have their deleterious damage to living cells; such chain reactions are terminated by antioxidants. This indicates that antioxidant activity was markedly higher in *Lawsonia inermis* as compared to *Emblica officinalis* when diluted in methanol. Moreover, methanol proved to be a more effective reagent to produce ($P < 0.05$) accurate results for determining antioxidant activity in *Lawsonia inermis* compared to ethanol reagent; while ethanol and methanol reagents were equally effective to determine the antioxidant activity of *Emblica officinalis*.

**Figure 7** Antioxidant activity from (20%) of henna and amla determined from 20% ethanol and methanol extracts (see online version for colours)




## 5 Discussion

To sum up, the phytochemical analysis results of two medicinally important plants such as henna (*Lawsonia inermis*) and amla (*Emblica officinalis*) using ethanol and methanol

reagents is very important. The following paragraph is a summary of results on phenolic compounds, antioxidant activity, carbohydrates, reducing sugars, total proteins, flavonoids, and tannins determination. The results showed in *Emblica officinalis* that the phenolic compounds were 41.9281 µg/ml and 41.3381 µg/ml when ethanol and methanol reagents were used, respectively; while in *Lawsonia inermis* samples, the phenolic compounds were 27.1191 µg/ml and 35.2316 µg/ml under ethanol and methanol dilutions, respectively (Yasoubi et al., 2007). Similarly, antioxidant activity was markedly higher in *Lawsonia inermis* 3.218 µg/ml under methanol dilution and decreased in *Lawsonia inermis* to 2.411 µg/ml under ethanol dilution. In *Emblica officinalis*, the antioxidant activity showed similarity (2.838 µg/ml and 2.851 µg/ml) under ethanol and methanol reagents, respectively. Two medicinally important plants henna (*Lawsonia inermis*) and amla (*Emblica officinalis*) were tested for their methanol and ethanol-based extracts to determine their antioxidant activity (Prieto et al., 1999).

The carbohydrates were higher in *Emblica officinalis* (10.076 µg/ml) under methanol dilution and decreased to 9.086 µg/ml under ethanol extract (Nielsen, 2010). To determine total carbohydrate contents of the prepared sample by phenol-sulphuric acid method reported by Nielsen (2010). In *Lawsoni ainermis*, carbohydrates were 6.547 µg/ml and 6.276 µg/ml under ethanol and methanol extracts, respectively. Sugar reduction was higher in *Emblica officinalis* ethanol extracts (0.573 µg/mL) than in methanol extract (0.180 µg/ml) and the reduction in *Lawsonia inermis* was 0.259 µg/ml and methanol extract (0.035 µg/ml). The reducing sugar contents of prepared samples were determined by the reported method, Miller (1959). The total proteins were markedly higher (0.511 µg/ml and 0.506 µg/ml) in methanol and ethanol extracts of *Emblica officinalis*; while in *Lawsonia inermis*, total proteins were (0.420 µg/ml and 0.245 µg/ml) under methanol and ethanol extracts, respectively (Lowery et al., 1951).

The flavonoids in the plant *Emblica officinal* were 0.796 µg/ml and 0.668 µg/ml in the extracts of methanol and ethanol; the flavonoids in *Lawsonia inermis* were 0.261 µg/ml and 0.154 µg/ml respectively. The tannins were exceptionally higher (4.455 µg/ml) in ethanol extracts of *Emblica officinalis*; declined to 1.336 µg/ml in methanol reagent (Tamilselvi et al., 2012); while in *Lawsonia inermis*, tannins were 1.292 µg/ml under methanol extract and declined to 0.664 µg/ml in ethanol dilution.

## 6 Conclusions

The variation is determined values of phenolic compounds under the influence of ethanol and methanol reagents were exceptionally greater in the case of *Lawsonia inermis* samples while negligible for *Emblica officinalis*. Antioxidant activity was higher in *L. inermis* in methanol extract; while ethanol and methanol reagents were equally effective to determine the antioxidant activity of *E. officinalis*. Carbohydrates were higher in *E. officinalis* than *L. inermis*, where methanol extract showed more accurate results for carbohydrates in *E. officinalis* and Ethanol extract in *L. inermis*. Methanol reagent proved to be more effective to determine accurate contents of reducing sugars in *L. inermis* while ethanol extract for *E. officinalis*. Ethanol extract was ineffective to provide accurate total protein determination in *L. inermis* but effective for *E. officinalis*. The flavonoids were higher under methanol extract compared to ethanol suggested that both the test plants methanol extract resolute accurate values for flavonoids. For the

determination of tannins in *E. officinalis* plants ethanol extract was more effective to provide accurate determination; while in *L. inermis* methanol extract showed accuracy for tannins determination.

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