Quantitative and Qualitative Analysis of *Eucalyptus grandis*, *Moringa oleifera* Leaf, *Punica granatum* Pericarp and *Syzygium aromaticum* Dried Bud

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ABSTRACT

Phytochemical compounds produced in plants are responsible for their biological activities. Medicinal plants have been known to be the backbone of traditional medicine long before recorded history and have been used as supplements to improve health and also wellbeing. In traditional medicine, plants have been used to prevent and cure infectious conditions and diseases caused by pathogenic microorganism.Therefore this study aimed to investigate the phytochemicals present in the leaves of *Eucalyptus grandis* and *Moringa oleifera* pericarp of *Punica granatum* and dried bud of *Syzygium aromaticum* crude plant extract.Oualitative and quantitavie phytochemical screening of aqueous and methanolic crude extract of four selected plant material were carried out using standard procedure with little or no modification.Result shows that phytochemicals such as tannins, terpenoids, phenols, steroids and anthocyanin were present in both aqueous and methanolic crude extracts of the plant materials. Maximum amount of carbohydrate (761µg/ml) and flavonoids were noted in methanolic extract of *P.granatum* pericarp. Total tannins (633µg/ml), proteins (323µg/ml) were noted in the aqueous extract of *S.aromaticum*. Highest amount of phenols (494µglml) were noted in aqueous extract of *M.oleifera.*This finding concluded that the selected plants materials consist of many useful bioactive compounds which are principal reason for a plant to possess medicinal properties.

Keywords: Phytochemical analysis, *Eucalyptus grandis, Moringa oleifera,Punica granatum, Syzygium aromaticum*

1 INTRODUCTION

Medicinal plants have different chemical compounds that work together synergistically and catalytically in order to produce a tremendous effect that exceed the total activity of the individual compounds.Plants are the oldest source of pharmacologically active compounds and have been used for medicinal purpose, natural dye, detergent, cosmetic and functional foods for centuries (Milovanovic et al., 2009; Raskin et al., 2002). Substances derived from plants remain the basis for a large production of many convectional drugs used for treatment of diseases for example Vinblastine an anti-cancer drug originated from *Catharatus roseus* plant (Gilani et al., 1999; Gopal, 2006).

Plants are able to produce a large number of chemical compounds that are accumulated in fruits likes pomegranate and apple, vegetables like onions, herbs, nut, legume and spices which include clove and tumeric (Doughari & Obidah, 2008; Doughari et al., 2009), high concentrations of which may protect against free radical damage (Suffredini et al., 2004; Zhang et al., 2015). These non-nutrient plant chemical compounds or bioactive components can be referred to as phytochemicals or phytoconstituents (Doughari, 2012).Plants containing beneficial phytochemicals may also supplement the needs of the human body by acting as natural antioxidants (Boots et al., 2008). Over the ages, these plant products which are derived from plant parts such as stem, barks, leaves, fruits, pericarp and seeds have been part of phytomedicine, thus indicating that any part of a plant may contain important active compounds (Inavova et al., 2005).
Eucalyptus grandis is commonly known as flooded gum or rose gum in Queensland (Boland et al., 2006). It belongs to the family of Myrtaceae and indigenous to Australia.Nowadays, *Eucalyptus grandis* is cultivated in several parts of the world including Argentina, Brazil, Ethiopia, India, Kenya, South Africa, Tanzania and Zambia (Orwa et al., 2009). Studies havereported that its essential oil exhibit properties such as anticancer,

antioxidant, antiviral and antimicrobal activity (Soyingbe et al., 2013 and Nagpal et al., 2017).

Moringa oleifera is the most widely cultivated species of the genus *Moringa* and it belongs to the family Moringaceae. *Moringa oleifera* is a drought resistance tree, indigenous to the southern foothills of the Himalayas in northwestern India where it is commonly known as drumstick.Rich in compounds that contain simple sugar such as rhamnose called glucosinolates and isothiocyanate (Fahey et al., 2001; Bennett et al., 2003;Dubey et al., 2013). It exhibit characteristics such as antihelmintic (Bondya et al., 2002), antibiotic, detoxifier, antipytrtic (Singh and Kumar, 1999), acrid, bitter (Oliveira, 1999) outstanding immune builder and also used in many developing countries to treat malnutrition and malaria.

Punica granatum commonly known as pomegranate is one of the first domesticated fruit that has been cultivated in different countries throughout the world for medicinal purposes (Morton, 1987).It is native to modern-day Iran, extending to neighboring countries such as Afghanistan, Pakistan and to northern India. (Morton,1987). It is an ancient fruit with distinctive characteristics such as antioxidant, anti-cancer, antiinflammatory, bactericidal, and fungicidal properties.

Syzygium aromaticum which is also known as clove is one of the most valuable sweet-flavored spice found in the family of Myrtaceae, genus *Syzygium* and *aromaticum* species that has been used for centuries as food preservative and for many medicinal purposes.Clove is indigenous to Maluka Islands in Indonesia but nowadays is cultured in several parts of the world. Cloves are commercially harvested in places like Brazil, Bangladesh, India, Pakistan, Tanzania, Madagascar and Sri Lanka. Commercially, it is used for the production of clove oil which has many pharmacological properties like, anti-oxidant, antiinflammatory (Kim et al., 1998), anti-carcinogenic agents, anti-mutagenic(Miyazawa et al., 2003) anti-viral (Hessein et al., 2000), anti-parastic (Yang etal., 2003), anti-thrombotic (Srivastava and Malhotra, 1991) and antiseptic.

2 MATERIALS AND METHODS

2.1Collectionand Preparation of Samples

The fresh leaves of *Eucalyptus grandis*, *Moringa oleifera*, pericarp of *Punica granatum* and dried bud of *Syzygium aromaticum* were collected from Botanical garden Noida sector 37.

The plant materials were gently removed, washed thoroughly under running tap, followed by distilled water to remove the particles. Sliced into pieces using a sterile knife, oven dry at 35ºC for 3-4 days and were powdered by using an electric mixer. For this experiment, maceration and decoction were carried out for aqueous and methanolic extraction respectively. For methanolic extraction, 5g of each dry plant materials was weighed and were added separately to 50 ml of methanol solution in the ratio of 9:1 (9 parts of methanol: 1 part of water). The mixture was placed in water bath and heated at 60°C for 1 hour. The mixture content was then filtered using Whatman No. 2 filter paper and the crude drug extract was obtained (Joshi *et al*., 2008; Mithun et al., 2015).In the case of aqueous extraction,same grams of sample was weighed and were added to water in a different conical flask in the ratio of 1:10 and placed in a mechanical shaker with intermittent shaking for 24hrs. The solution obtained was filtered using Whatman No. 2 filter paper followed by centrifugation and the resulting filtrate was stored for the succeeding experimentations.

2.2 Phytochemical Screening

Phytochemical test were carried out on the aqueous and methanolic extract of *E. grandis, M. oleifera, P. granatum* and *S.aromaticum* using standard procedure to identify the constituents as described by (Edeoga *et al*., 2005; Trease and Evans, 2005; Harbone, 1999) with little or no modification.

2.2.1 Test for Alkaloid: To 2ml of the extract was boiled with dilute hydrochloric acid and the mixture was filtered and to the filtrate a few drops of Mayer's reagent was added. A cream or white colour precipitate produced immediately indicates the presence of alkaloids.

2.2.2 Test for Tannins: 2ml of each extract was added separately to 4ml of water and a few drops of 0.1% $FeCl_3$ were added to the extracts Formation of blue color indicated the presence of hydrolysable tannins and formation of brownish black color indicates the presence of catechol or condensed tannin (Joshi *et al*.,

2011).

2.2.3 Test for Carbohydrate(Fehlings test): 2 mg dry extract was dissolved in 1ml of distilled water and 1ml of Fehling's (A+B) solution was added, shook and heated on a water bath for 10 minutes. The brick red precipitate formed confirmed the test

2.2.4 Flavonoid (Alkaline reagent test): 2ml of extracts was treated with few drops of 20% sodium hydroxide solution formation of intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

2.2.5 Protein Test: To 3ml of various extracts, few drops of 4% sodium hydroxide was added along with 1% copper sulphate. The formation of reddish violet colour indicates the presence of proteins.

2.2.6 Terpenoids (Salkowaski test): 5ml of various extracts was taken in different test tubes. To each of them 2ml of chloroform was added, along with it 3ml of concentrated sulphuric acid was added slowly to form a layer. A red brown color formed at the interface indicated the test as positive for triterpenoids (Edeoga *et al*., 2005).

2.2.7 Test for Phlobatannins: An aqueous extract of the plant sample was boiled with 1% aqueous hydrochloric acid and a deposition of a red precipitate will show positive but there were no colour changes which indicate the absence of phlobatanins (Sofowora, 1993).

2.2.8 Test for Saponins: 1ml of the extract was added to 20ml of distilled water in a test tube and was shaken vigorously for 15 minutes. Formation of the foamy layer indicated the presence of saponins (Kokate, 1991).

2.2.9 Hydroxyanthraquinone Test: To 1ml of the extract, added a few drops of 10% potassium hydroxide solution. The Formation of red color confirmed the test.

2.2.10 Cardiac Glycosides (Keller-Killani test)**:** 2ml of glacial acetic acid was added to 5ml of the extracts containing a drop of ferric chloride solution followed by the addition of 1ml of concentrated (Edeoga *et al*., 2005).

2.2.11 Phenol (Ferric Chloride test): To 1ml of the extract 5% ferric chloride solution are added. A dark green colour indicates the presence of phenolic compound (Mace, 1963).

2.2.12 Steriod(Salkowaski test): To 2ml of extracts, 2ml of chloroform was added along with 2ml of concentrated sulphuric acid slowly through the side of the test tube.

2.2.13 Anthocyanin (Alkaline reagent test): 2ml of extracts was treated with few drops of 20% sodium hydroxide solution formation of intense yellow colour indicates the presence of anthocyanin.

2.3 Quantitative phytochemical analysis

2.3.1Determination of Reducing Sugar by Dinitrosalicyclic acid (DNS) Method

Various concentrations of glucose was used to determine the calibration curve of carbohydrate. The concentration of glucose used was 1mg/ml. The solution was properly stirred using a magnetic stirrer in order to obtain a homogenous solution. Varied volume of glucose ranging from 0-3ml was pipetted into test tubes and the volume was made up to 3ml with distilled water. 2ml of DNS reagent was added to the test tubes and was kept in boiling water for 15 minutes. After 15 minutes the test tubes were cool to room temperature. Blank was prepared by the addition of 3ml of distilled water and 2ml of DNS reagent. Absorbance was read at 540nm wavelength. The result obtained was used to plot a standard curve of carbohydrate and estimate the concentration of the unknown.

2.3.2 Estimation of flavonoids by Aluminum (111) chloride colorimetric assay

Using a pipette, varying amount of distilled water was pippeted into a test tube followed by quercetin. The concentration of quercetin used was 1mg/ml. To the test tubes, 0.3ml of sodium nitrate (5% (w/v) $NaNO_2$) was added and was allowed to stand for 5 minutes to react. After which 0.3ml ofaluminium chloride(10% (w/v) $AlCl_3$) were added to the test tube and was left to react for 1 minute. 2ml of sodium hydroxide (1M NaOH) was added and the mixtures were shaken before reading the absorbance at 510nm. Blank was prepared by the addition of 7.4ml of distilled water, 0.3ml of $NaNO_2$, 0.3ml of (10% (w/v) $AlCl_3$) and 2ml of 1M NaOH while the samples were prepared the same way with the standard but quercetin was replaced with the extracts. A standard curve was constructed with the optical density at 510nm against the concentration of quercetin and the total flavonoids of the samples were estimated from the standard curve.

2.3.3 Estimation of Total Phenolic Contents by Folin-Ciocalteu method

The calibration curve used for the estimation of total phenolic content of the extracts was obtained by using varying concentration of gallic acid solution. The concentration used was 100µg/ml. Varied volume of distilled water ranging from 450µl to 500ul was added to the test tubes followed by gallic acid solution 0 to 50µl to make it up to 500µl. To each test tube, 2.5ml ofFolin-Ciocalteureagent (FCR) (1ml of 0.2N FCR was added to 1ml of distilled water) was added and allow to stand at room temperature for 5 minute. 2ml of 75% sodium carbonate was then added and incubated at room temperature for 1hour 30 minutes. Blank was prepared by the addition of 500µl of distilled water, 2.5ml of FCR and 2ml of 75% sodium carbonate while the samples were prepared the same way with the standard but gallic acid was replaced with extracts. Absorbance was read at 765nm wavelength and the result obtained was used to plot a standard phenolic curve and estimate the concentration of the unknown.

2.3.4 Estimation of Protein Concentration by Folin and Lowry's Method

The calibration curve used for protein determination was obtained using varying concentration of Bovine Serum Albumin (BSA). The concentration of the BSA stock solution used was 1mg of Bovine serum albumin (BSA) in 1ml distilled water. Varied volume of distilled water was pipetted into test tubes followed by BSA. 4.5ml of alkaline copper reagent was added to the test tube and allowed to stand at room temperature for 10 minutes. To the test tube 500µl of Folin-Cwas added and the mixture was shook vigorously and allowed to stand for 30 minutes. Blank was prepared by the addition of 1ml of distilled water, 4.5ml of alkaline copper reagent and 500µl of Folin-C. Absorbance was read at 660nm wavelength. The result obtained was used to plot a standard protein curve and estimate the concentration of the unknown.

2.3.5 Estimation of Tannin by Van Burden and Robinson Method

Various concentration of tannic acid was used to obtained the calibration curve of tannin and estimate the concentrated of tannin in the samples. The concentration of tannic acid used was 1mg/ml of distilled water. The solution was properly stirred by using a magnetic stirrerin order to obtain homogenous solution. Varied volume of distilled water ranging from 4.5 to 5ml was added into test tubes followed by tannic acid. 2ml reagent was added to the test tubes and absorbance was read within 10 minutes at 605nm using a spectrophotometer. The result obtained was used to plot standard tannin curve and estimate the concentration of the unknown.

3RESULTS

3.1QualitativePhytochemical Screening

Table 1 shows the phytochemicals present in the aqueous extracts of *E grandis* leaves, *M. oleifera* leaves, pericarp of *P. granatum* and flowering bud of *S. aromaticum.*The major phytochemicals present in the four extracts were Carbohydrate, Tannins, Phenol, Steriods, Terpenoids and Hydroxyanthraquinone. Saponin was present in all except *E. grandis*. Cardiac Glycosides, Alkanoids, Flavonids, Fat and oil and protein were present in two of the extracts while Phlobatannins was not detected in all.

Table 1: P**hytochemical analysis of aqueous extract of Clove, Moringa, Pomegranate and Eucalyptus**

Phytochemicals	Clove	Eucalyptus	Moringa	Pomegranate
Alkaloid	++	--	+	--
Anthocyanin	--	+	+	--
Carbohydrate	+++	+	+	+++
Glycoside	+	+	-	+++
Fat and oil	--	--	--	+++
Flavonoids	++	+	+++	--
Phenol	+++	+++	+	+++
Phlobatanin	--	--	--	--
Protein	--	+	--	+
Saponin	+++	--	+++	+++
Steriod	+	+	+++	++
Tannin	+++	+++	+++	+++
Triterpenoid	+	+	+++	++
Hydroxyanthraquinone	+	+	+	+++

Key: +++highly positive, ++ moderately positive, +mildly positive, -- negative

Table 2 shows the phytochemicals present in the methanolic extracts of *E. grandis* leaves, *M. oleifera* leaves, pericarp of *P. granatum* and dried bud of *S. aromaticum.* Unlike aqueous extracts, carbohydrate and hydroxyanthraquinone were not present in all the methanolic extracts. The major phytochemicals present in the four extracts were Tannins, Phenol, Steriods, Triterpenoids and Anthocyanin. Saponin was highly present in eucalyptus and pomegranate but showed trace amount in clove and moringa. Hydroxyanthaquinone and carbohydrate were also present in all except in *Moringa oleifera*. Cardiac glycosides, Alkanoids, Flavonids, fat and protein were present in two of the extracts while Phlobatannins was not detected in all.

Table 2: Phytochemical analysis of methanolic extract of Clove, Moringa, Pomegranate and Eucalyptus

Phytochemicals	Clove	Eucalyptus	Moringa	Pomegranate
Alkaloid	+	--	+	++
Anthocyanin	--	+	+++	++
Carbohydrate	+++	++	--	+++
Glycoside	+	+	-	+++
Fat and oil	--	--	--	+++
Flavonoids	--	+	+++	++
Phenol	+++	+++	++	+++

Phlobatanin	--	--	--	--
Protein	--	+	--	+
Saponin	+	+++	+	++
Steriods	+++	+	+++	+++
Tannin	+++	++	+++	+++
Triterpenoid	+++	++	++	+++
Hydroxyanthraquinone	+	+	+	+++

Key: +++highly positive, ++ moderately positive, +mildly positive, -- negative

3.2 Quantitative determination of carbohydrate, flavonoids, phenols, proteins and tannins in of the 4 selected medicinal Plants

The result for the total carbohydrate content of eucalyptus, clove, moringa and pomegranate extracts showed that methanolic extract of*P. granatum*had the highest value (761µg/ml). The least value (118µg/ml)was noted in the aqueous extract of *M. oleifera*.

Total flavonoids content of eucalyptus, clove, moringa and pomegranate extracts showed that the highest amount of flavonoids was reported in the methanolic extract of *P. granatum* with 686µg/ml. The least value of flavonoids (248.5µg/ml) was observed in *M. oleifera*.

Table 3 clearly indicated that the highest amount of phenols was reported in aqueous extract of *M. oleifera*and least amount of 411.5µg/ml w/v extract was found inmethanolic extract of*M. oleifera*

When the proteins concentrations were analyzedaqueous extract of *S. aromaticum* showed the highest value of 323µg/ml w/v of the extract and the least amount of 127µg/ml was observed in aqueous extract of *E. grandis*.

Finally, total tannins content were determined for the aqueous and methanolic extracts and the tannins were in the range of 50-635µg/ml w/v of the extracts. The aqueous extract of*S. aromaticum* showed the highest value of tannins (633µg/ml) and least amount was observed in the aqueous extract of *M. oleifera*.

Table 3:Show the results obtained from the quantitative analysis of aqueous extracts of all the selected medicinal plants

Plant source	**Carbohydrates (µg/ml)**	**Flavonoids (µg/ml)**	**Phenols (µg/ml)**	**Protein (µg/ml)**	**Tannins (µg/ml)**
Eucalyptus grandis	329	281	460	127	163
Moringa oleifera	118	270	494	201	53
Punica granatum	428	323	482	322	416
Syzygium aromaticum	402	296	485	323	633

Table 4: Show the results obtained from the quantitative analysis of methanolic extracts of all the selected medicinal plants

Plant source	Carbohydrates (µg/ml)	Flavonoids (µg/ml)	Phenols (µg/ml)	Protein (µg/ml)	Tannins (µg/ml)
Eucalyptus grandis	299	431	490	222	477
Moringa oleifera	297	248.5	411.5	203	356
Punica granatum	761	686	482.5	302	533
Syzygium aromaticum	367	483	485.5	312	539

4 Discussion

Phytochemical analysis of dried bud of *S. aromaticum*, pericarp of *P. granatum*, leaves of *E. grandis* and *M. oleifera* have shown presence of steroids, alkanoids, saponin, tannin, phenol, glycosides and others which are principal reason for a plant to possess medicinal properties. Flavonoids were detected in most of the extracts except in aqueous extract of pomegranate and methanolic extract of clove. Analysis in the present study is similar to the report of Shailash (2015) who revealed presence of tannin, saponin, terpenoid in both aqueous and alcoholic extract of clove whereas flavonoids and cardiac glycoside tested negative in alcoholic extract. In contrast to Shailash (2015) finding, cardiac glycoside is presence in both aqueous and methanolic extract of clove. In addition, another study found carbohydrates, tannins, steriods, saponins, flavonoids and alkanoids absence in methanolic and aqueous extracts of clove which is also in contrast to the present study (Jyothiprabha and Venkatachalam, 2016).

According to Roopalatha and Vijay (2013) preliminary phytochemical screening of moringa using a single solvent had been reported. Roopalatha and Vijay (2013) revealed that glycoside and protein are absence in the aqueous extract of moringa while hydroxyanthraquinone, saponin, carbohydrate tannin, steroids were presence in aqueous and ethanolic extracts of moringa which is similar to this work. In contrast to Roopalatha and Vijay finding, carbohydrate is absence in our methanolic extract and saponin occurs in trace amount. Pinal *et al*. (2014) has equally reported that alkaloids, flavonoids, carbohydrates, steriods, saponins, tannins and terpenoids are found in different solvent extracts of moringa which is also similar to this findings. In this analysis, alkanoids were tested to be positive in methanolic extract of pomegranate, aqueous extract of clove and moringa but negative in *E. grandis*. Closely in accordance with this finding is the study of Kannaiyan *et al* who reported the presence of flavonoids, carbohydrates, steriods, phenols, glycosides, tannins and terpenoids in the ethanolic pericarp extract of pomegranate (Kannaiyan *et al*., 2012). Glycosides, steriods, anthocyanin, carbohydrate, hydroxyanthraquione, phenol and tritepenoids were found in trace amount in aqueous extract of eucalyptus while they are moderately presence in the methanolic extract. In addition, saponins were highly presence in the extract.These differences might be attributed to factors like climate, percentage humidity, nutrition, chronological age of the plant, time of harvest, storage and solvent used for the extraction.

Secondary metabolites from plantsorigin might increase the stability of the phytochemical constituents thereby reducing the rate of its undesired adverse effects, and giving it an antagonistic effect (WHO, 2003; Gurib-Fakin, 2006; Chintamunnee and Mahomoodally, 2012; Shohawon and Mahomoodally, 2013). For instance, a single plant may contain saponin with bitter substances that enhances nutrient absorption and metabolism and also possess anti-inflammatory compounds that reduce swellings and pain, flavonoids that act as antioxidant or free radical scavengers and chelate metals that causes free radical production, tannins that act as natural antibiotics, diuretic substances that enhance the elimination of waste products and toxins, phenolic compounds that can act as an antiseptic, antibacterial and anti-fungi against pathogenic organism in human and alkaloids that enhance mood, give a sense of well-being and promote the discharge of mucus from the respiratory track.

5 Conclusion

This finding concluded that the selected plants materials consist of many useful bioactive compounds which are principal reason for a plant to possess medicinal properties and can serve as a great potential for treatment of infectious diseases andmanufacturing of new drugs.

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