

Original Research Article

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## Screening of Phytochemical Components of Leaf Extracts

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### ABSTRACT

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Phytochemicals are chemicals of plant origin, produced through primary or secondary metabolism of plants. Phytochemicals have become of great interest owing to their diverse applications. In the study five plant sources available locally and abundantly were selected to screen phytochemicals available in their leaves for testing their anti-microbial property for textiles. Leaf extracts were prepared using 70% ethanol and distilled water. The extracts were assessed for the presence of various classes of phytochemicals by applying standard tests and also total phenolic content of each extract was estimated. Results revealed that all the extracts contained tannins and phenolic compounds significantly. Flavonoids were poorly present in Amla leaf extract and significantly in Babul leaf extract. Alkaloids and saponin were poorly present in all extracts except moderate presence in Amlaleaf extract. Terpenoids were totally absent in all except Babul leaf extract. Ethanol extract of Amla leaf extract exhibited the highest Total Phenolic Content i.e. 156.79 mg/g of dry material followed by Babul leaf extract 117.99 mg/g. Among distilled water extracts Babul leaf extract exhibited highest i.e. 106.05 mg/g followed by Amla leaf extract i.e. 100.27 mg/g.

### Introduction

India is well known all over the world for its ayurvedic treatment since prehistoric time. A wide range of medicinal plant parts like root, stem, flower, fruit, twigs exudates and modified plant organs have been used for extraction of raw drugs Chandra M.(2013), Manikandan *et al.*, (2016). The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body Selvakumar Sivagnanam *et al.*, (2016). These

chemicals are termed as phytochemicals. Phytochemicals are chemicals of plant origin, produced by plants through primary or secondary metabolism. They generally have biological activity in the plant host and play an important role in plant growth. They are naturally present in the plants to defend themselves against various pathogenic microbes by showing the antimicrobial activity by inhibiting or killing microbes. Medicinal plants are richest bio-resource of drugs in traditional system of medicine and it also responsible for different colours, flavors

and smell of plant Pradeep *et al.*, (2014). Phytochemicals have recently become great interest owing to their versatile application Pushpa Ruwali *et al.*, (2019), Saranraj *et al.*, (2016). Phytochemicals play an important role in various preparations like food, cosmetics, pharmaceutical, flavours and agrochemical etc. as antimicrobial agents as well as antioxidants. Phytochemical screening is very important in identifying new sources of therapeutically and industrially important compounds like Alkaloids, Flavanoids, Phenolic Compounds, Saponins, Steroids, Tannins, Terpenoids etc. Akindele *et al.*, (2007). They confer plants with odour (terpenoids), pigmentation (tannins and quinines), and flavor (capsacin) Mallikharjuna *et al.*, (2007). They are a part of plant naturally defense system. These bioactive components are said to be responsible for the

antimicrobial effects of plant extracts in vitro Ankita Sood *et al.*, (2012). The mode of action of plants producing antimicrobial effects on selected textile materials can be better investigated if the active ingredients are identified and characterized Vastrad *et al.*, (2016). There is worldwide realization that any plant known for a particular bio-efficacy should be explored. Muhammad Gulfraz *et al.*, (2011). All the plant sources selected for the study were available locally and in abundant. The leaves of the plants were used for procuring the extract for testing the presence of phytochemical compounds.

### Materials and Methods

Information about the plants selected for the study

S. No	Name of the plant source	Botanical name	Family	Part used
1	Amla	<i>Phyllanthus emblica</i>	Phyllanthaceae	Leaves
2	Periwinkle	<i>Catharanthus roseus</i>	Apocynaceae	Leaves
3	Lemon	<i>Citrus limon</i>	Rutaceae	Leaves
4	Acacia	<i>Vachellia nilotica</i>	Fabaceae	Leaves
5	Drumstick	<i>Moringa oleifera</i>	Moringaceae	Leaves

### Processing of plant sources

The leaves of the plants i.e. Amla, Periwinkle, Lemon, Babul and Drumstick were collected from the plants grown in the campus of the Vasant Rao Naik Marathwada Krishi Vidyapeeth, Parbhani. The leaves were then washed thoroughly in distilled water and dried on a cloth to drain out excess water. Further drying of the leaves was done in the shade. The dried leaves were then ground into fine powder. Powder was then sieved through the sieve with mesh no. 150 to separate the unwanted residue and to get fine powder.

### Extract preparation

Soaked 30 gm of the plant source powder in 60 mL distilled water and in 70:30, Ethanol:

distilled water mixture for 24 hours at room temperature. Centrifuged on 5000 RPM and filtered the supernatants. The residue was subsequently mixed with 25 mL of respective solvent and the process repeated for next 24 hours. The final extract obtained was filtered through Whatman filter paper.

### Phytochemical analysis

Qualitative phytochemical screening was done for the identification of various classes of phenolic constituents, i.e. alkaloids, flavonoids, terpenoids, saponins etc. Different chemical tests performed for detecting these phenolic groups. The qualitative results are expressed as phytochemicals were (+++) for significantly present, (++) for moderately present, (+) for poorly present, (-) for absent.

### **Test for Tannins and Phenolic compounds**

(a) Ferric chloride test: One mL of extract was separately stirred with 10 mL of distilled water and then filtered. A few drops of 5%  $\text{FeCl}_3$  were added to the filtrate. Blue-black or blue-green colouration or precipitation was taken as an indication of the presence of tannins.

(b) Lead acetate test: Three mL of 10% lead acetate solution was added to 1 mL of extract. Appearance of bulky white precipitate confirmed the presence of phenolic compounds.

### **Test for Flavonoids**

(a) Ammonia test: A few drops of 1%  $\text{NH}_3$  solution was added to 1 mL of the extract in a test tube. A yellow coloration was observed for the presence of flavonoids.

(b) Sodium hydroxide test: Few drops of 20%  $\text{NaOH}$  solution was added to 1 mL of extract. On addition of  $\text{HCl}$ , the changed yellow colour of the extract turned to a colourless solution that depicted the presence of flavonoids.

### **Test for alkaloids**

(a) Dragendorff test: To 1 mL of extract, few drops of Dragendorff's reagent were added. A prominent yellow precipitate indicated presences of alkaloids.

(b) Wagner test: Few drops of Wagner's reagent were added by the side of test tube to 1 mL of extract. A reddish-brown precipitate confirmed the test as positive.

### **Test for saponins**

Foam test: About 1 mL of the extract was boiled in 20 mL of distilled water in a water bath and

filtered, 10 mL of the filtrate was mixed with the 5 mL of distilled water and mixed vigorously for 15 min to form a stable persistent froth. The presence of froth after 5 min was taken as an indication of presence of saponins.

### **Test for Terpenoid**

Salkowski test: One mL of each extract was mixed with 0.5 mL of chloroform and 1 mL of concentrated  $\text{H}_2\text{SO}_4$  was carefully added to form a layer. A reddish brown colouration of the interface formed showed positive results for the presence of terpenoids.

### **Estimation of Total Phenolic Content (TPC)**

Total Phenolic Content was determined using the Folin-Ciocalteu Assay method (Singleton and Rossi 1965) using Gallic acid as the reference standard. To one mL of solvent extract 100  $\mu\text{L}$  of Folin-Ciocalteu reagent, was added and incubated at room temperature for 3 minutes then added 2 mL of 10%  $\text{Na}_2\text{CO}_3$  solution to the mixture.

The resulting solution was incubated for 90 minutes at room temperature under dark conditions, the absorbance was measured at 765 nm using the UV-Visible Spectrophotometer. Total Phenolic Content is expressed as gallic acid equivalent (GAE) in milligrams per gram of sample.

### **Results and Discussion**

Table 1 showed presence of phytochemicals in Amla (*Phyllanthus emblica*) leaf extract. Amla (*Phyllanthus emblica*) leaf extracts in ethanol as well as in distilled water showed tannins and phenolic compounds significantly, flavonoids poorly, alkaloids and saponins moderately while terpenoids were absent

**Table.1** Phytochemical screening of Amla (*Phyllanthus emblica*) leaf extract

S.No	Phytochemical tests	Solvents	
		Distilled water	Ethanol
<b>1</b>	<b>Test for tannins and phenolic compounds</b>		
<b>a</b>	Ferric chloride test	+++	+++
<b>b</b>	Lead acetate test	+++	+++
<b>2</b>	<b>Test for flavonoids</b>		
<b>a</b>	Ammonia test	+	+
<b>b</b>	Sodium hydroxide test	+	+
<b>3</b>	<b>Test for alkaloids</b>		
<b>a</b>	Dragendorff test	+	+
<b>b</b>	Wagner test	++	++
<b>4</b>	<b>Test for saponins</b>		
<b>a</b>	Foam test	++	++
<b>5</b>	<b>Test for terpenoids</b>		
<b>a</b>	Salkowski test	-	-

+++ = Significantly present ++ = moderately present + = Poorly present - = Absent

**Table.2** Phyto- chemical screening of Lemon (*Citrus lemon*) leaf extract

S.No	Phytochemical test	Solvents	
		Distilled water	Ethanol
<b>1</b>	<b>Test for tannins and phenolic compounds</b>		
<b>a</b>	Ferric chloride test	+	-
<b>b</b>	Lead acetate test k	+++	+++
<b>2</b>	<b>Test for flavonoids</b>		
<b>a</b>	Ammonia test	-	-
<b>b</b>	Sodium hydroxide test	-	-
<b>3</b>	<b>Test for alkaloids</b>		
<b>a</b>	Dragendorff test	+	+
<b>b</b>	Wagner test	+	+
<b>4</b>	<b>Test for saponins</b>		
<b>a</b>	Foam test	+	+
<b>5</b>	<b>Test for terpenoids</b>		
<b>a</b>	Salkowski test	-	-

+++ = Significantly present, + = Poorly present, - = Absent

**Table.3** Phytochemical screening of drumstick (*Moringaoleifera*) leaf extract

S no	Phytochemical tests	Solvents	
		Distilled water	Ethanol
<b>1</b>	<b>Test for tannins and phenolic compounds</b>		
<b>a</b>	Ferric chloride test	+	+
<b>b</b>	Lead acetate test	-	-
<b>2</b>	<b>Test for flavonoids</b>		
<b>a</b>	Ammonia test	-	-
<b>b</b>	Sodium hydroxide test	-	-
<b>3</b>	<b>Test for alkaloids</b>		
<b>a</b>	Dragendorff test	+	+
<b>b</b>	Wagner test	-	-
<b>4</b>	<b>Test for saponins</b>		
<b>a</b>	Foam test	+	+
<b>5</b>	<b>Test for terpenoids</b>		
<b>a</b>	Salkowski test	-	-

+ = Poorly present - = Absent

**Table.4** Phytochemical screening of babul (*Acacia nilotica*) leaf extract

S. no.	Phyto- chemical tests	Solvents	
		Distilled water	Ethanol
<b>1</b>	<b>Test for tannins and phenolic compounds</b>		
<b>a</b>	Ferric chloride test	+++	++
<b>b</b>	Lead acetate test	+++	+++
<b>2</b>	<b>Test for flavonoids</b>		
<b>a</b>	Ammonia test	+++	+
<b>b</b>	Sodium hydroxide test	-	-
<b>3</b>	<b>Test for alkaloids</b>		
<b>a</b>	Dragendorff test	+	-
<b>b</b>	Wagner test	+	-
<b>4</b>	<b>Test for saponins</b>		
<b>a</b>	Foam test	-	-
<b>5</b>	<b>Test for terpenoids</b>		
<b>a</b>	Salkowski test	+	+

+++ = Significantly present + = Poorly present - = Absent

**Table.5** Phytochemical screening of Periwinkle (*Catharanthus roseus*) leaf extract

S no	Phytochemical test	Solvents	
		Distilled water	Ethanol
<b>1</b>	<b>Test for tannins and phenolic compounds</b>		
<b>a</b>	Ferric chloride test	++	-
<b>b</b>	Lead acetate test	+++	+++
<b>2</b>	<b>Test for flavonoids</b>		
<b>a</b>	Ammonia test	-	-
<b>b</b>	Sodium hydroxide test	-	-
<b>3</b>	<b>Test for alkaloids</b>		
<b>a</b>	Dragendorff test	+	+
<b>b</b>	Wagner test	+	+
<b>4</b>	<b>Test for saponins</b>		
<b>a</b>	Foam test	+	+
<b>5</b>	<b>Test for terpenoids</b>		
<b>a</b>	Salkowski test	+	+

+++ = Significantly present ++ = moderately present + = Poorly present - = Absent

**Table.6** Total phenolic content of leaf extracts

Sl. No.	Plant source	TPC (mg/g of dry material)	
		Distilled water	Ethanol
<b>1</b>	Lemon	19.75	41.82
<b>3</b>	Drumstick	27.29	55.47
<b>4</b>	Periwinkle	18.93	33.85
<b>5</b>	Babul	106.05	117.99
<b>6</b>	Amla	100.27	156.79

Table 2 exhibits presence of phyto-chemicals in Lemon (*Citrus lemon*) leaf extract. Lemon (*Citrus lemon*) leaf extracts in ethanol as well as distilled water contained tannins and phenolic compounds significantly, alkaloids and saponins poorly while, flavonoids and terpenoids were absent.

Table 3 indicates phytochemical screening of Drumstick (*Moringa oleifera*) leaf extract. Drumstick (*Moringa oleifera*) leaf extracts in ethanol as well as distilled water contained tannins poorly but phenolic compounds were absent, alkaloids and saponins were poorly present while flavonoids and terpenoids were absent.

Table 4 shows presence of phytochemical in Babul (*Acacia nilotica*) leaf extract. In ethanol and distilled water extracts of Babul (*Acacia nilotica*) leaf, tannins and phenolic compounds were significantly present. Extract in distilled water showed flavonoids significantly but ethanol extract contained flavonoids poorly. Alkaloids were poorly present in water extract and absent in ethanol extract. In both the extracts terpenoids poorly present while saponins were absent.

Table 5 exhibits phytochemical contents of Periwinkle (*Catharanthus roseus*) leaf extract. Both distilled water and ethanol extracts of periwinkle leaf contained tannins and

phenolic compounds significantly, alkaloids, saponins and terpenoids poorly while flavonoids were absent.

Table 6 showed Total Phenolic Content of leaf extracts in distilled water and ethanol. Highest phenolic content was found in Amla leaf extract in ethanol i.e. 156.79mg/g of dry leaf powder followed by Babul leaf extract in ethanol (117.99 mg/g). Distilled water extract of Babul leaves had TPC 106.05 mg/g and Amla leaf extract had 100.27 mg/g.

Total Phenolic Content of Drumstick, Lemon and Periwinkle leaf extracts obtained using ethanol were 55.47, 41.82 and 33.85 mg/g of material respectively while TPC of the extracts of them taken out in distilled water were 27.29, 19.75 and 18.93 mg/g of dry weight respectively. It is observed that Total Phenolic Content of the leaf extract taken out in ethanol was more than the TPC of the respective leaf extracts taken out in distilled water.

In conclusion the extracts of leaves of all the selected plants contained tannins and phenolic compounds significantly. Flavonoids were poorly present in Amla leaf extract and significantly present in Babul leaf extract. Alkaloids and saponin were poorly present in all extracts except moderate presence in Amla leaf extract. Terpenoids were totally absent in all except Babul leaf extract. Ethanol extracts of Amla leaf extract exhibited the highest Total Phenolic Content i.e. 156.79 mg/g of dry material followed by Babul leaf extract 117.99 mg/g.

Among distilled water extracts Babul leaf extract exhibited highest i.e. 106.05 mg/g followed by Amla leaf extract i.e. 100.27 mg/g. Total Phenolic Content of the leaf extract taken out in ethanol was more than the TPC of the respective leaf extract taken out in distilled water.

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