



# PHYTOCHEMICAL SCREENING AND ANALYSIS OF SECONDARY METABOLITITES IN PLANT EXTRACTS FROM OLDENLANDIA CORYMBOSA (L).

J. SESHADRI.,M.Sc.,M.Phil.,

Ph.D., Research Scholar,

PG & Research Department of Botany,

Government Arts College,

C.Mutlur, Chidambaram

Dr. R. ARUNADEVI.,M.Sc.,M.Phil.,Ph.D.,

Assistant Professor,

PG & Research Department of Botany,

Government Arts College,

C.Mutlur, Chidambaram.

## ABSTRACT:

The term of medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities. Medicinal plants are the “backbone” of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis. Plants synthesize secondary metabolites which include alkaloids, flavonoids, saponins, terpenoids, steroids, glycosides, tannins, volatile oils etc., The glassware and media were sterilized in an autoclave at 15psi for 20 minutes, at 120 °C. Plant was thoroughly cleaned, dried The powdered plant material was mixed with 70% aqueous-methanol and stored at room temperature for 5 days. The extract obtained was dried and stored in an airtight container at 4°C. Polyphenols are valuable plant constituents for the scavenging of free radicals because of their phenolichydroxyl groups. This, together with the obtained results, suggests that as the amount of

polyphenolic compounds increases, the antioxidant activity also increases. The result shows the synthesis of phytochemical components and justifies the medicinal values.

**KEYWORDS:** Distilled Water, Autoclave, Sodium Hydroxide, H<sub>2</sub>SO<sub>4</sub>, Bismuth nitrate.

## INTRODUCTION:

Medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. Besides that these plants play a critical role in the development of human cultures around the whole world. The Indian sub-continent has a very rich diversity of plant species in a wide range of ecosystems. There are about 17,000 species of higher plants, of which approximately 8,000 species, are considered medicinal and used by village communities, particularly tribal communities, or in traditional medicinal systems, such as the Siddha and Ayurveda. The use of traditional medicine and medicinal plants in most developing countries, as a basis for the maintenance of good health, has been widely observed by UNESCO, 1996. Medicinal plants frequently used as raw materials for extraction of active ingredients which used in the synthesis of different drugs. Medicinal plants are an integral component of research developments in the pharmaceutical industry.

Medicinal plants have two types of metabolites one of which is primary and another secondary. Primary metabolites are involved in all process directly but secondary metabolites do not involve directly into metabolic processes. It can increase all metabolic and catabolic reaction. (Nupur joshi et al. 2013); Various parts of this plant have medicinal effect and reported to possess potent emetic, narcotic activities (Krishnamurthy A, 1969). Plant based drugs continue an important source of beneficial agents because of the accessibility, comparatively inexpensive cost and non-toxic nature when associated to modern medicine (Agbor and Ngogang, 2005).

The secondary metabolites producing natural drugs and controlled botanical pesticides. Always the higher plants producing primary and secondary metabolites in normal vitally important reproduction of plants (Herbert 1981; Duke 1992). Even though our body is safeguarded by natural antioxidant defense, there is always a demand for antioxidants from natural sources (Rimbach *et al.*, 2005). Antioxidant activity is the common assay used and widely accepted by researchers as an anticancer indicator (Tsai *et al.*, 2005). The addition of antioxidants is a method of increasing shelf life, especially of lipids and lipid-containing foods. Bioavailability is the proportion of the nutrient that is digested, absorbed and metabolized through normal pathways. Bioavailability of each and every polyphenol differs however there is no relation between the quantity of polyphenols in food and their bioavailability in human body.

## MATERIALS AND METHODS

### STERILIZATION OF GLASSWARE

Glassware were soaked overnight in cleaning solution and washed thoroughly with running tap water. They were then cleaned with detergent solution and rinsed several times with tap water and finally in distilled water and air dried. The glassware and media were sterilized in an autoclave at 15psi for 20 minutes, at 120 ° C.

### PLANT MATERIALS

The *Oldenlandia corymbosa.L* whole plant were collected from Government siddha medical college, herbal garden, Arumbakkam, Chennai, Tamilnadu, during july 2019 and it was taxonomically identified and authenticated as *Oldenlandia corymbosa.L*. A voucher specimen was deposited in the herbarium for future reference (Ref.No. MB/2017/Rub-24).

### PREPARATION OF EXTRACTS

The *Oldenlandia corymbosa.L* whole plant was thoroughly cleaned, dried under the shade and coarsely powdered. The polyphenolic rich extract was prepared according to a previously reported method Kumarappan *et al.*(2012). The powdered plant material was mixed with 70% aqueous-methanol and stored at room temperature for 5 days. After 5 days, it was filtered and the solvent was evaporated. The residue was dissolved in water, and the aqueous layer was washed with petroleum ether several times until a clear upper layer of petroleum ether was obtained. The lower layer was then treated with ethyl acetate containing glacial acetic acid(10 mL/L). Extraction of the polyphenols was performed for 36 h at room temperature, and the combined ethylacetate layer was then concentrated. The residue was lyophilised. The extract obtained was dried and stored in an airtight container at 4°C. The yield of the dry poly-phenolic extract was 30.5% (w/w). The dried extract was dissolved in Milli-Q water and used for further study.

## PHYTOCHEMICAL SCREENING

The aqueous methanol extract of *Oldenlandia corymbosa.L* were subjected to phytochemical screening to determine the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, glycosides, saponins and polyphenols using standard procedures (Harborne 1998; Trease and Evans 1983).

### TEST FOR TANNINS

0.5 g of the dried powder of *Oldenlandia corymbosa.L* were boiled in 20 ml of water in a test tube and then filtered using No. 1 Whatmann filter paper. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

### TEST FOR SAPONINS

5g of fresh aqueous methanol extract of *Oldenlandia corymbosa.L* was weighed accurately, boiled in 20 ml of distilled water for few minutes and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The formation of 2cm layer of foam was observed indicates the presence of saponins.

### TEST FOR FLAVONOIDS

#### a. Alkaline Reagent test:

To 1ml of the aqueous methanol extract of *Oldenlandia corymbosa.L*, a few drops of dilute sodium hydroxide was added. A intense yellow colour was observed indicates the presence of flavanoids.

#### b. Lead acetate test:

A few drops of lead acetate (10%) solution was added to 1 ml of each aqueous methanol extract results in the formation of yellow precipitate indicates the presence of flavanoids.

### TEST FOR TERPENOIDS

#### a. Salkowski test

Five ml aqueous methanol extract of *Oldenlandia corymbosa.L* were mixed with 2 ml of chloroform and 3 ml of concentrated  $H_2SO_4$  was added carefully to form a layer. The reddish brown colour was formed at the interface indicates the presence of terpenoids.

## TEST FOR GLYCOSIDES

The aqueous methanol extract of *Oldenlandia corymbosa.L* was hydrolyzed with Hydrochloric acid for few hours on a water bath and then the hydrolysate was subjected to Bornbager's test for the presence of glycosides.

### a. Keller-Killani test

2 ml of aqueous methanol extract of *Oldenlandia corymbosa.L* was mixed with glacial acetic acid followed by the addition of few drops of 5%  $\text{FeCl}_3$  and concentrated  $\text{H}_2\text{SO}_4$ . The reddish brown color appears at the junction of the two layers and the upper layer turns bluish green in color which indicates the presence of glycosides.

## TEST FOR ALKALOIDS

### a. Dragendroffs Test

#### Preparation of Dragendroffs reagent:

8 g of Bismuth nitrate [ $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ ] was dissolved in 20ml of  $\text{HNO}_3$  (solution A) and 2.72g of Potassium iodide in 50ml of  $\text{H}_2\text{O}$  (solution B). The solution A & B were mixed and allowed to stand for deposition of  $\text{KNO}_3$  Crystals. The Supernatant was discarded and made up to 100ml with distilled water.

#### Procedure:

1 ml of each aqueous methanol extract of *Oldenlandia corymbosa.L* was mixed thoroughly with 2ml of  $\text{HCl}$ . To this acidic medium 1ml of dragendroff's reagent was added, orange or red precipitate was observed indicates the presence of alkaloids.

### b. Mayers test

#### Preparation of Mayers reagent:

1.36g of Mercuric chloride was dissolved in 60ml of distilled water (solution A) and 5g of Potassium iodide (solution B) in 10ml of water. The solution A & B were mixed and diluted to 100 ml with distilled water.

**Procedure:**

To 1 ml of aqueous methanol extract of *Oldenlandia corymbosa.L* 0.2 ml of dilute HCl and few drops of Mayers reagent was added. The yellow color precipitate was observed indicates the presence of alkaloid.

**TEST FOR POLYPHENOLS**

To 5 ml of aqueous methanol extract of *Oldenlandia corymbosa.L* 1ml of FeCl<sub>3</sub> (1%) and 1ml K<sub>3</sub> (Fe (CN) <sub>6</sub>) (1%) was added. The appearance of radish blue color indicated the presence of polyphenols.

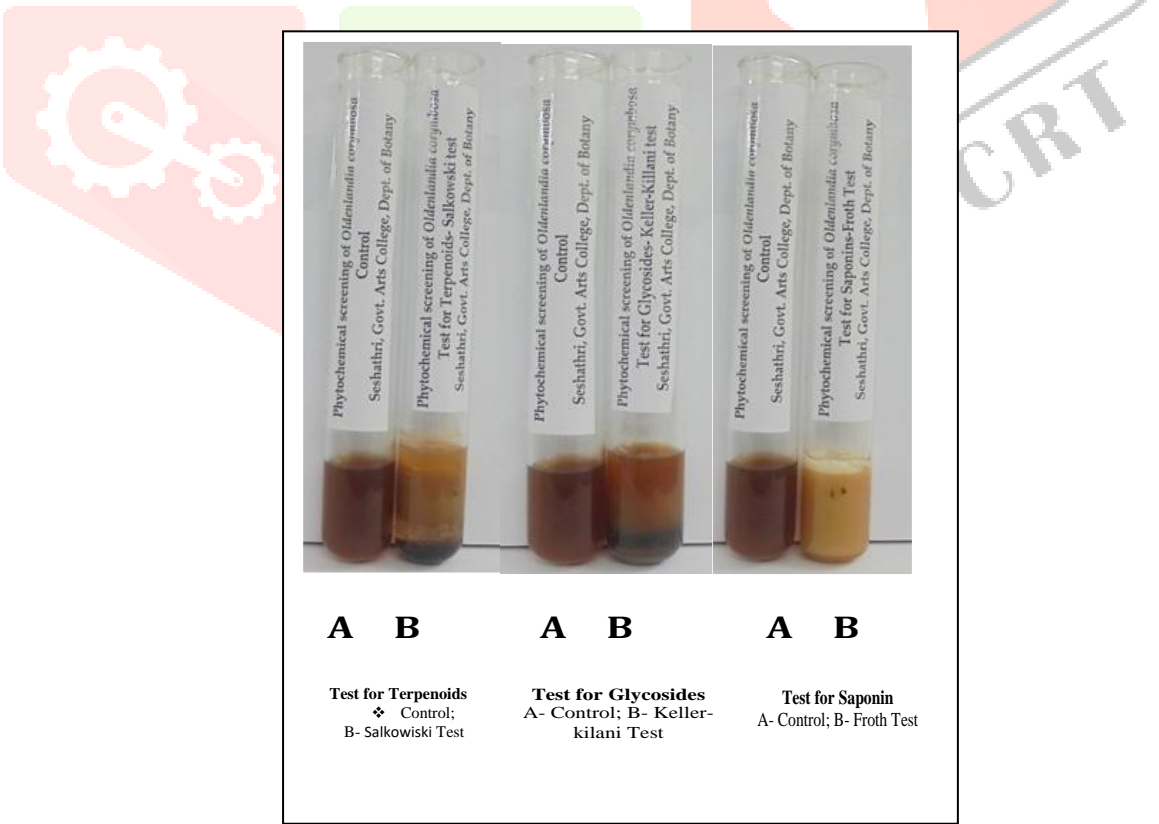
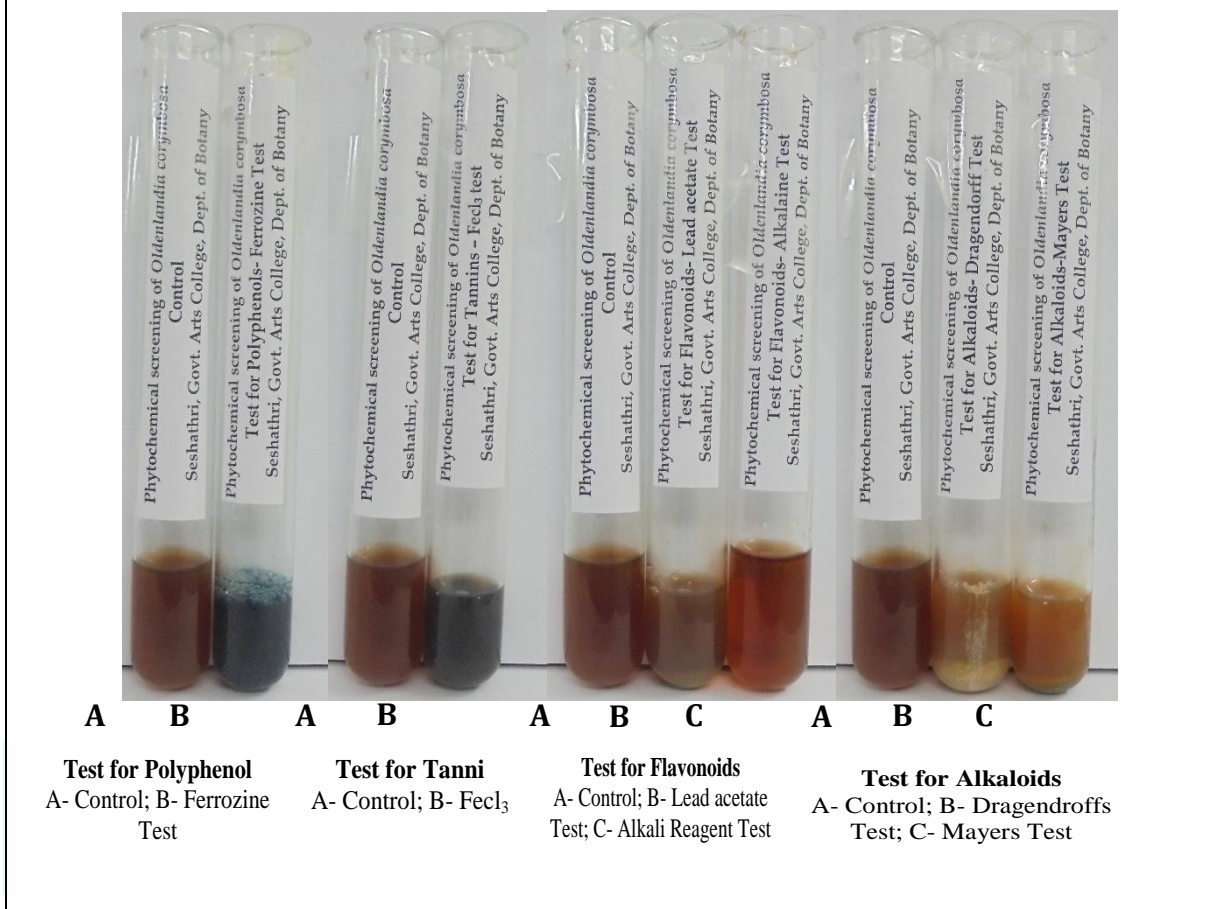
**RESULT AND DISCUSSION**

The phytochemical screening of aqueous methanol extract of *Oldenlandia corymbosa.L* studied presently showed the presence of alkaloids, flavonoids, polyphenol, terpenoids, and absence of glycosides and tannin (Table -1 and Fig-2).

**Table-1. Phytochemical screening of aqueous methanol extract of *Oldenlandia corymbosa.L***

S/No.	Constituents	Aqueous methanol extract of <i>Oldenlandia corymbosa.L</i>
1.	Alkaloids-Dragendroffs reagent	+
	Alkaloids- Mayers Test	
2.	Flavonoids- Alkali reagents	+
3.	Tannin- Fecl <sub>3</sub> test	+
4.	Saponins- Frothing test	+
5.	Terpenoids - Nollers test	+
6.	Glycosides- Keller-Killiani Test	-
7.	Polyphenols-Ferrozine	+

**Fig-2. Phytochemical screening of aqueous extract of *Oldenlandia corymbosa*.(L)**



The present study on *Oldenlandia corymbosa* (L). extract was concerned percentage of secondary metabolites produced. The alkaloids using by pain-killers, antimicrobial, stimulants, muscle relaxants, anaesthetics, antimicrobial, anti-diabetic, anti-cancerous, anti-HIV, antioxidants etc. (Nupur joshi *et al.*,2013). The phytochemical screening of polyphenolic extract of *Oldenlandia corymbosa.L* studied presently showed the presence of all metabolites except Glycosides. Phytochemical components are known to be biological active compounds and they are control for different activities such as antioxidant, antimicrobial, antifungal, and anticancer (Hossain and Nagooru, 2011; Suresh and Nagarajan, 2009). Our phytochemical analysis of the total extracts of *Oldenlandia Corymbosa* (L).revealed similar results to previous findings. Phytochemical ingredients such as alkaloids, glycosides, reducing sugar, flavonoids, tannins, saponins, and various organic compounds are secondary metabolites of medicinal plants that serve as defense mechanism against many microorganisms and insects (Bonjar *et al.*, 2004). Secondary metabolites found in plants are methanol and terpenoids which represent the main antimicrobial agents. Similarly, aromatic compounds such as phenolic acids, alkaloids and flavonoids have also been identified as antimicrobial agents (Siddiqui *et al.*, 2009).The presently study on antimicrobial activity compounds occurs in significant levels.

## CONCLUSION

In conclusion, *Oldenlandia Corymbosa*(L). contain potential Secondary metabolites and phytochemical components that may be used for the development of pharmaceuticals as a therapy against various diseases. This plant extracts could serve as possible sources of experimental antimicrobial and antioxidant agents. In which studies are necessary to develop a method for the fractionation and identification of polyphenols and to determine the most active antioxidant compounds in the polyphenolic extract.



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