International Journal of Research Instinct
(Peer-reviewed Journal)

ISSN ONLINE 2348-2095
Research Article

Efficacy of *Oldenlandia umbellata* root extracts against respiratory tract pathogens

[1] Sujatha R K and [2] Thenmozhi S

[1 & 2] *Asst. professor ,Dept. of Microbiology, Srimad Andavan Arts and Science college, (Autonomous),T.V. Kovil, Trichy.*

E mail: rkrksmoorthy@yahoo.com

Abstract

Respiratory diseases are the major cause of death in the developing countries. To overcome the health problem people prefer allopathic medicine which cause side effects. In the modern world people were shifted to traditional system of medicine. Because, it cure the diseases and also not cause any side effects. In the present study, the attempts are made to control the respiratory causative bacteria with traditional medicinal plant, *Oldenlandia umbellata*. Alcohol and aqueous extracts of the plant showed antimicrobial activity against the tested respiratory pathogens. The phytochemical analysis showing the presence of alkaloid, terpenoids, flavones, flavanoids, tannins, amino acids and these substances are responsible for antibacterial activity. This study scientifically proves the importance of plant products in development of a potent antibacterial agent. Further research will be carried to find all bioactivity of *Oldenlandia umbellata* root extracts.

Keywords

Respiratory pathogens, Oldenlandia umbellata, phytochemical compounds, Thin layer chromatography (TLC)

Introduction

India has a rich heritage of traditional knowledge and is a birth place to several important time-honored systems of health care like Ayurveda, Siddha and Unani. Plants are potent biochemical factories and have been possess the components of phytomedicine. The medicinal effects of plants are due to metabolites especially secondary compounds produced by plants. The metabolites are present in the plant for the defense of themselves, which we can make use for human welfare. Terpenoids, special nitrogen metabolite, non-protein amino acids, amines, cynogenic glycosides, glucosinolates, alkaloids and phenol are the major components of the plant

metabolites and plays vital role in curing disease.

Diseases occur through various reasons like pathogenic Microbes, deficiency, hereditary etc., and considered to be a major threat to human society. Now-a-days, pathogenic microbial diseases like respiratory tract infection, which include asthma, bronchitis, sneezing, cold, cough, gastrointestinal infections like diarrhea, dysentery, ulcer and metabolic disorders in the form of stress, diabetics, cancer etc., are frequently encountering humans. Incidence of the diseases may vary from, cough, mild stomach upset to the dangerous cancer. Usually antibiotics are a group of medicines that are used to treat infections caused by germs (bacteria and certain parasites). It can create the side effects like Nausea, vomiting, memory loss, headache, stomach pain, etc.

The amount of synthetic drug is even costless and the use of synthetic antimicrobial drugs had led to gradual emergence of populations of antibiotic-resistant bacteria. The systematic screening of antibacterial effects from plant extracts represents a continuous effort to find new potential compounds which act against multidrug resistant bacteria. This has pushed us for the search of newer antimicrobial agents from herbal medicinal sources. Herbal medicine are used for different purposes usually the act as a medicine in day today life to cure many aliments. The plant materials can be treated major health problems such as respiratory tract infection (*Oldenlandia umbellate*), gastrointestinal infection (*Terminalia belerica*), genitourinary tract infection (*Mangifera indica)* [1].

Oldenlandia umbellata is known as *Hedyotis umbellata*. This genus comprises of herbs and shrubs distributed in the tropical and subtropical regions of the world. About seventy species occur in India, some of which are used in medicine. The leaves and roots are considered expectorant and used in asthma of bronchitis [2] and the root powder has been subjected to clinical trails and it has been proved to be an efficacious remedy for various diseases, particularly in tuberculosis [3]. *Oldenlandia umbellata* are showing a maximum antibacterial activity [4]. Hence, the present study was conducted to screen antibacterial activity and phytochemical activity with roots of *Oldenlandia umbellata*.

Materials and Methods

Isolation and identification of isolates

The respiratory tract pathogens were isolated from the infected patients and specific colonies from selective and differential media were subjected to macroscopy and microscopical study. The biochemical tests (Indole test (I), Methyl Red test (MR), Voges Proskauer test (VP), Citrate Utilization test (C), Urease production test (U), Nitrate Reduction test (N), Cytochrome

Oxidase activity, Catalase test, etc) were conducted for further identification.

Microscopic observations like size, shape and motility availability of different morphology characters among microorganisms. Simple staining, gram staining and hanging drop methods were done to look for their shape.

Collection and process of plant material

Oldenlandia umbellata root was collected from the local market, shade dried and coarsely powdered by making use of mechanical blender. The powder was stored in an airtight container and was used for extraction.

Pharmacognostic studies

Organoleptic Character Evaluation [5].

Oldenlandia umbellata root was subjected to macroscopic studies which comprised of organoleptic characters of the drugs viz., colour, odour, appearance, taste, smell, texture.

Antibacterial study of plant extract

Preparation of water extract

200 gm of plant material was taken and extracted with water. To one part of the plant material six parts of water added and boiled till the total contents were reduced to one third and filtered. The filtrate obtained was evaporated to dryness, till it becomes paste form, this paste form of the extract was used for the study.

Extraction of plant materials

The plant material was air dried at room temperature (26^0 C) for 2 weeks, after which it was ground to uniform coarse powder. An ethanolic extract was prepared by soaking 250g of the dry powdered plant material in 750 ml of ethanol at room temperature for 72hours. The extract was filtered after 72 hours, first through a muslin cloth, then using whatman filter paper No. 42 (125 mm) and then through cotton wool. The extract was concentrated using a rotary evaporator with water bath set 40^0 C. the percentage yield of extracts were also calculated.

Column chromatography

The lower end of a glass column was plugged with glass wool. The material was poured on the glass wool and air bubbles released was trapped with the flat end of packed rod. The column was packed with wet silica gel by pouring the silica gel into the column in a stepwise manner. The side of the column was taped gently with a glass rod compaction of the particles. As silica gel settles, the column outlet was adjusted. The sample was drawn onto the absorbent and eluted with mixture ethanol. All fractions obtained were collected.

Quality analysis of secondary metabolites by thin layer chromatography

Preparation of TLC slide

TLC (Thin layer chromatography) plate was prepared by mixing silica gel –G in distilled water. Mixture is prepared in a colloidal form, and then it was poured and spread to the glass slide was as a thin layer.

Application of sample

Silica gel coated TLC slide was taken. Starting line was taken. Starting line was drawn 15mm above the lower edge using marking pencil. Plant extract was applied on the starting lines as spot by making use of capillary tube. All the extracts and fractions of the plant parts were applied in various plates. Spot was made and was allowed to cool room temperature.

Development of chromatogram

TLC slide was placed in a beaker saturated with solvent such as alcohol and water then chromatogram was allowed to run, developed at room temperature by allowing the solvent to ascend the specified distance.TLC plate was removed from the beaker and position of the solvent front was marked, solvent available in the plate was allowed to evaporate at room temperature.

Observation

TLC plate was observed in daylight initially. Iodine chamber was used as a spreading reagent. The distance of each spot to the point of application was recorded. RF values were calculated by making use of formula.

RF= Distance traveled by the solute / Distance traveled by the solvent

Phytochemical analysis of metabolites [6,7]

Powder material was extracted with various solvents in the order of increasing polarity and the extracts were subjected to phytochemical screening of metabolites like Steroids, terpenoids, flavonoids, tannins, cardiac glycoside, flavones, carbohydrates, proteins, aminoacids, lignin and inulin by standard methods.

Microbial Quality Control [8].

Medicinal plant materials normally carry a greater number of bacteria and molds after originating from the soil. Tests were conducted for the analysis of Total viable aerobic count (TVAC), Total Viable Count (TVC) and Test for Enteric pathogens (TEP).

Antimicrobial activity test

Test microorganisms

Klebseilla, Pseudomonas, Staphylococcus, Streptococcus

Preparation of discs

Known quantity of extracts was dissolved in DMSO and it was filtered sterilized by making use of syringe filter of pore size 0.22 µm. Sterile discs of 6 mm diameter (Hi-Media) were loaded with various concentrations of extracts and dried. Dried discs were stored in sterile containers till use. Solvent loaded discs were also prepared and used as negative control. Loaded Norfloxacin Hi-Media discs were used as positive control.

Preparation of inoculum

The isolates were inoculated in nutrient broth and incubated at 37^0Cfor 4 hours in a shaker (Orbitec ; Scigenics, India) and was used for anti - bacterial activity test and to look for the MIC of various extracts and fractions.

Determination of antibacterial activity [9].

Disc diffusion method was followed to determine the anti - bacterial activity of various extracts and fractions. Petri plates containing 20 ml of Mueller Hinton agar were seeded with 4 hours old fresh cultures. By making use of template drawn extracts loaded discs were dispensed on the solidified Mueller Hinton agar with test organisms.

Norfloxacin antibiotic disc obtained from M/s Hi-Media laboratories Ltd, Mumbai was used as a positive control and solvent loaded discs were used as a negative control. This was incubated at 37°C for 24 hours in an incubator (Rands SBC). The test was performed in triplicates. The zone of inhibition was measured by making use of Antibiotic zone scale (Hi - media).

Determination of Minimum Inhibitory Concentration[10]

Agar dilution method was used to find out Minimal Inhibitory Concentration[11]. Stock concentration of various plant extract was prepared by making use of DMSO .Methanol, in the ratio of 1:1 which in turn was diluted with equal volume of phosphate buffered saline . pH - 7. Muller Hinton Agar was prepared, sterilized and kept ready in molten condition. 20ml of the molten media was taken tubes. This mixture was swap carefully for complete mixing of extract and media and poured on to the plate.

After getting solidified it was inoculated with the test organisms and standard organisms. The plates were incubated at 37^0C for 24 hours. MIC was recorded based on the growth of the organisms.

Result and discussion

The medicinal plants contain active constituents that are used in the treatment of much human disease [12]. Many of the plant used in traditional medicine are readily available in rural areas

at relatively cheaper than modern medicine. Thus it is important to characterize different types of medicinal plant for their antioxidant and antimicrobial potential [13,14,15].

Organoleptic evaluation serves as an important criterion to categorize medicinal plant. It is based on sensory feature of the crude plant powder indicating its important diagnostic character [5]. Organoleptic studies of the root powder were performed to observe the macroscopic and morphology characters. Organoleptic nature of the root powder is found to be dark green in colour, bitter taste and aromatic odour, crystallin in texture

Quality of the extract plays a vital role in usage. Contaminations of microbes in plant extract if result it inhibit the efficiency of the powder. Therefore it is highly necessary to use without any contamination and required to check its microbial load. Such contamination were found to be absent in the presently studied plant. There is no enteric isolates, enumerated from the plant powder 50×10^{-2} CFU bacterial loads was found which were within the limits of ayurvedic pharmacopeia of India (Table-1).

TABLE -1 Microbial Limit Assay Of *Oldenlandia umbellata* (Root Powder)

S.NO	TEST ORGANISMS	MICROBIAL COUNTS CFU/g
1	Total aerobic bacteria	50×10^{-2}
2	Total fungal count	Nil
3	Total enteric bacteria	Nil
4	Total *E.coli*	Nil
5	*Salmonella*	Nil

The phytoconstituents of medicinal plant play a vital role in different healing property. Phytochemical evaluation was performed for qualitative detection of various chemical constituents which aid in tracing the presence of active entity that elicit a major pharmacology response. Aqueous and alcohol extract of *Oldenlandia umbellata* [16]responsible for antibacterial activity .Antimicrobial activity is due to the presence of secondary metabolites. These compound precipitate surface proteins of microorganism there by inhibit microorganism.

The present study result showed the presence of terpenoids, tannins, flavones, inulin, lignin in alcoholic extracts and terpenoids, tannins, flavones, inulin,lignin in aqueous extract respectively on the other hand, *Oldenlandia umbellata* ethanolic extract is further subjected to various fraction through column chromatography.Fraction-1, 2, 3 & 4 showed the presence of alkaloids,

flavonoids,terpenoids,tannins, flavones,aminoacids, inulin. It has been stated that the mechanism of the antimicrobial activity of the plant extract involves the inhibition of various cellular processes, increase in plasma membrane permeability and impairment of energy that involves in the synthesis of structural compounds in microbial cells [17].This preliminary phytochemical test were helpful in predicting the nature of drug and also useful for the detection of different constituents present. Flavonoids and tannins may be major group of compound that act as primary antioxidants or free radical activity scavenger.

Screening of antibacterial activity need properly identified strain of microorganism. Totally 4 strain were tested and are subjected to identification by making use of microscope, macroscopic and biochemical method (Table-2) that provide basic information about morphology of the isolates on different selective cum differential media (Table-3).

Isolates were named 1, 2, 3, 4 based on varies biochemical test isolated were indentified 1- *Klebsiella*, 2- *Pseudomonas*, 3- *Staphylococcus,* 4- *Streptococcus.* Disc diffusion method was adopted to screen antibacterial activity of plant extracts. Aqueous and alcoholic extracts of *Oldenlandia umbellate* root were collected and subjected to antimicrobial screening. Roots of alcoholic extract of *Oldenlandia umbellata* showed best activity (15mm) against *Streptococcus* 800µg/disc concentration. Aqueous extract produced 12mm against *Streptococcus* at 800µg/disc concentration (Table-4).

Results revealed that the extract produced at least 6mm of zone of inhibition against the entire organism tested at 200µg/ disc concentration. Results of this study have shown that both aqueous and ethanolic extract of *Oldenlandia umbellata* possess antibacterial activities. This observation may be due to the presence of bioactive substance in both the roots extract. Ethanolic extract was more efficacious than the aqueous extract (Table-4).

Further column chromatography is employed to purify secondary metabolites from ethanolic extracts. Four fractions of solutions obtained with different colours,Fraction-1dark green colour, Fraction-2 is light dark green. Fraction-3 green in color and Fraction-4;light green. Then fractions were subjected to antimicrobial analysis Fraction-1 showed 15mm for *Streptococcus* at 800mg/disc, Fraction-2 showed 18mm for *Staphylococcus* at 800mg/dics,Fraction-3 showed 14mm for Staphylococcus at 800mg/disc, Fraction-4 showed 16mm for *Streptococcus* 800mg/disc.

As per above statement fraction 1,2,3,4 showed best results for specific organisms. This observation in this study reveals that the variation occurred may be due to the ability of the active

ingredient present in *Oldenlandia umbellata* that highly dissolve more in ethanol than in water (Table-7, plate-2).

TABLE -2

Identification of Pathogens Based on Morphological and Biochemical Test

Identification Of Pathogens Based On Biochemical Test

(Carbohydrates Fermentation Test)

S.NO	SUGARS	*Klebseilla*	*Pseudomonas*	*Staphylococcus*	*Sterptococcus*
1.	Glucose	No colour change	No colour change	No colour change	No colour change
2.	Sucrose	Slightly colour change/ Gas bubbles	No colour change	No colour change	No colour change
3.	Fructose	Colour change	Colour change	No colour change	No colour change
4.	Dextrose	Colour change/ Gas bubbles	Colour change	No colour change	Colour change/ Gas bubbles
5.	Lactose	Colour change	No colour change	No colour change	No colour change
6.	Galactose	No colour change	Colour change	No colour change	No colour change
7.	Xylose	Gas bubbles/Colour change	Gas bubbles	No colour change	No colour change

TABLE- 3

Identification Features of Pathogens Based on Selective Media

S.NO	MEDIUM	*Klebseilla*	*Pseudomons*	*Staphylococcus*	*Streptococcus*
1.	*Klebseilla* base agar	White colour colonies	No growth	No growth	No growth
2.	Nutrient agar	White colour colonies	No growth	White colour colonies	White colour colonies
3.	Blood agar	Non haemolytic	Non haemolytic rough colonies	Non haemolytic	No growth
4.	EMB agar	No growth	No growth	Pink Colour colonies	Pink Colour colonies
5.	Pseudomonas base agar	No growth	Green colour colonies	No growth	No growth
6.	Mannitol salt agar	No growth	No growth	Red colour colonies	No growth

TABLE -5

Antibacterial Activity Of *Oldenlandia umbellata*Water Extract

S.NO	ORGANISM	CONCENTRATION OF EXTRACT (µg/disc) ZONE OF INHIBITION IN(mm)					
		POSITIVE	NEGATIVE	200 µ	400µ	600µ	800µ
1	*Klebseilla*	25	-	10	10	12	22
2	*Pseudomonas*	12	-	4	5	10	12
3	*Staphylococcus*	25	-	10	12	16	18
4	*Streptococcus*	18	-	10	10	11	12

TABLE -6

Antibacterial Activity Of O*ldenlandia umbellata* Ethanolic Extract

S.NO	ORGANISM	CONCENTRATION OF EXTRACT (µg/disc) ZONE OF INHIBITION IN mm					
		POSITIVE	NEGATIVE	200 µ	400µ	600µ	800µ
1	*Klebseilla*	21.66	-	5	11	12	22
2	*Pseudomonas*	12	-	5	6	10	12
3	*Staphylococcus*	22	-	6	8	10	12
4	*Streptococcus*	16	-	10	11	12	15

TABLE -7

Antibacterial Activity Of *Oldenlandia umbellata* root Extract

Fraction-1

S.NO	ORGANISM	CONCENTRATION OF EXTRACT (µg/disc) ZONE OF INHIBITION IN mm					
		POSITIVE	NEGATIVE	200 µ	400µ	600µ	**800µ**
1	*Klebseilla*	24±12	-	10±54	10±45	12±44	22±08
2	*Pseudomonas*	11±076	-	4±33	5±67	10±44	12 ±54
3	*Staphylococcus*	23±12	-	10±23	12±89	16±33	18±33
4	*Streptococcus*	17±132	-	10±11	10±09	11±12	14±22

Antibacterial Activity Of *Oldenlandia umbellata* root Extract

Fraction-2

S.NO	**ORGANISM**	**CONCENTRATION OF EXTRACT (µg/disc) ZONE OF INHIBITION IN mm**					
		POSITIVE	**NEGATIVE**	**200 µ**	**400µ**	**600µ**	**800µ**
1	*Klebseilla*	11±56	-	6±11	10±07	10±55	14±22
2	*Pseudomonas*	12±076	-	-	-	-	5 ±22
3	*Staphylococcus*	23±12	-	6±22	6±89	10±45	18±454
4	*Streptococcus*	11±132	-	9±33	10±76	12±34	14±55

Antibacterial Activity Of *Oldenlandia umbellata* root Extract

Fraction-3

S.NO	**ORGANISM**	**CONCENTRATION OF EXTRACT (µg/disc) ZONE OF INHIBITION IN mm**					
		POSITIVE	**NEGATIVE**	**200 µ**	**400µ**	**600µ**	**800µ**
1	*Klebseilla*	10±	-	6±	10±	10±	14±
2	*Pseudomonas*	6±	-	-	-	-	5 ±
3	*Staphylococcus*	21±	-	6±	6±	10±	22±
4	*Streptococcus*	15±	-	9±	12±	13±	26±

Antibacterial Activity Of O*ldenlandia umbellata* root Extract

Fraction-4

S.NO	ORGANISM	CONCENTRATION OF EXTRACT (µg/disc) ZONE OF INHIBITION IN mm					
		POSITIVE	NEGATIVE	200 µ	400µ	600µ	800µ
1	*Klebseilla*	6±09	-	6±22	10±22	10±56	14±09
2	*Pseudomonas*	-	-	-	-	-	5 ±99
3	*Staphylococcus*	23±12	-	6±43	6±12	10±78	18±243
4	*Streptococcus*	22±132	-	12±22	14±34	18±98	24±45

In this work plant extract revealed good antimicrobial activity. Ethanolic extracts produced more zone of inhibition against different pathogen when compared to aqueous. This would suggest that plant ethanolic extract may consider as best replacement for the current antibiotic therapy. Various constituents were responsible for creating antimicrobial property. Flavonoids reduce the motility of the bacterium. These components were estimated from the plant material differ from different parts of the country. Among extract *Oldenlandia umbellata* fractions obtained by column chromatography showed best results followed by crude ethanol and aqueous extracts.

Acknowledgment

All the authors profoundly thank the management of Srimad Andavan Arts and Science College, (Autonomous), T.V. Kovil, Trichy, for the sustainable support and success of the present study. Also my sincere thanks to Head and staff members, Department of Microbiology for providing constant laboratory access.

References

[1] Seydal, P. and Dorneburg, H.2006. *Establishment of in vitro plants, cell and tiisue cultures Oldenlandia umbellata for the production of cyclic peptides. Plants cell Tiss. Org. cult.,* 85:247-255.

[2] Kirtikar, D. M., & Goldthwait, D. A. (1974). *The enzymatic release of O6-methylguanine and 3-methyladenine from DNA reacted with the carcinogen N-methyl-N-nitrosourea. Proceedings of the National Academy of Sciences,*71(5), 2022-2026.

[3] Purushothaman KK, Saradha K and NarayanasamiV.1972. *Imbural(Oldenlandia umbellata). J.Res. Indian Med*; vol 7(3): 37.

[4] Thomas E, Shanmugam J, Rafi MM. 1999 .*Invitro anibacterial activity of certain medicinal plants of kerala biomedicine;* 19(3): 185-190.

[5] Kokate, T. G., Svensson, B. E., & Rogawski, M. A. (1994). *Anticonvulsant activity of neurosteroids: correlation with gamma-aminobutyric acid-evoked chloride current potentiation. Journal of Pharmacology and Experimental Therapeutics,* 270(3), 1223-1229.

[6] nonymous. *Indian Herbal pharmacopeia. IDMA Mumbai 1998; 1:30-7*

[7] Lala PK, *Practical Pharmacognosy, 1, Vallabh Prakashan, New Delhi,* 1981, 86-95.

[8] Anonymous. *Indian Pharmacopoeia, Vol I & II. Government of India, Ministry of Health and Family Welfare, The controller of publications, Civil lines, Delhi-1996.*

[9] Bauer*, AW., Kirby, W., Sherris, TS., and Turek, M. 1966. Antibiotic susceptibility testing by a standardized single disc method. Amer J of Clin Pathol 36: 493 – 496*

[10] Kowser, MM., and Fatema, N. 2009. *Determination of MIC and MBC of selected azithromycin capsule commercially available in Bangladesh. The ORION Medical Journal* 32(1):619-620.

[11] National Committee for Clinical Laboratory Standard (NCCLS) (*1993). Performance standards for antimicrobial susceptibility testing,* 15(14): 100-156.

[12] Campo, J. D., & Amiot, M. J. (2000). *Antimicrobial effect of rosemary extracts. Journal of Food Protection,* 63(10), 1359-1368.

[13] Mothana, R. A., & Lindequist, U. (2005*). Antimicrobial activity of some medicinal plants of the island Soqotra. Journal of ethnopharmacology,* 96(1), 177-181.

[14] Kim, J. K., Park, K. J., Cho, K. S., Nam S. W., Park, T. J., & Bajpai, R. (2005). *Aerobic nitrification–denitrification by heterotrophic Bacillus strains.Bioresource Technology,* 96(17), 1897-1906.

[15] Wojdyło, A., Oszmiański, J., & Czemerys, R. (2007). *Antioxidant activity and phenolic compounds in 32 selected herbs. Food chemistry,* 105(3), 940-949.

[16] Hurdle, J. G., O'Neill, A. J., Chopra, I., & Lee, R. E. (2011). *Targeting bacterial membrane function: an underexploited mechanism for treating persistent infections. Nature Reviews Microbiology*, 9(1), 62-75.

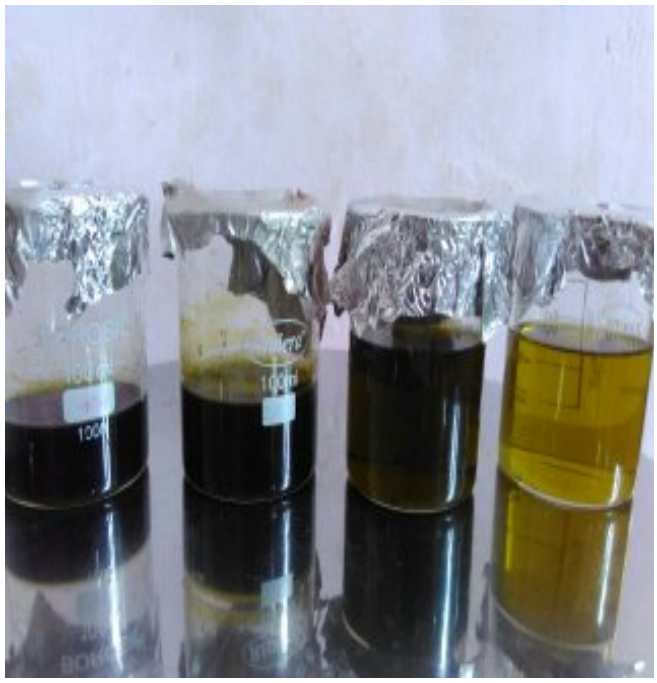
[17] De Wals, P., Rusen, I. D., Lee, N. S., Morin, P., & Niyonsenga, T. (2003). *Trend in prevalence of neural tube defects in Quebec. Birth Defects Research Part A: Clinical and Molecular Teratology,* 67(11), 919-923.

PLATE-1

Oldenlandia umbellata

PLATE-3

COLOUMN CHROMOTOGRAPHY WITH FRACTION



THIN LAYER CHROMOTOGRAPHY


TLC (COLD EXTRACT)
ETHANOL SOLVENT