ISSN: 2320-9267

Indian Journal of Pharmaceutical and Biological Research (IJPBR)

Journal homepage: www.ijpbr.in

RESEARCH ARTICLE

Evaluation of Phytochemical and HPLC analysis of *Crotalaria angulata*, *Momordica cymbalaria*, *Senna hirsuta* and *Indigofera linnaei* and it's biological applications

Vasanth Nayagam[1*], Joelri M. Raj[2], Thiraviadoss Dons[2]

ABSTRACT

To encompass a quantitative evaluation of phytochemical analysis and HPLC analysis of ethanol extracts of *Senna hirsuta* ; *Indigofera linnaei; Crotalaria angulata* and *Momordica cymbalaria* leaves were experimented to have broad analysis on presence of bioactive components. The phytochemical tests showed the bioactive compounds in *Senna hirsuta* ethanolic extracts with Steroids, Glycosides, Anthraquinones, Saponins Glycosides, Flavonoids and Terpenoids. In *Indigofera linnaei,* ethanolic extracts of this plant contain Steroids, Glycosides, Saponins, Glycosides and Terpenoids, Anthraquinones, Tannins, Flavonoids and Saponins are absent for this plant. Test for Steroids, Anthraquinones, Tannins and Terpenoids are strongly present in the plant of *Crotalaria angulata*. The strong presence of Steroids, Glycosides, Tannins, Terpenoids, Saponins foam in the plant of *Momordica cymbalaria*. The effects of ethanolic extracts of Anti-Bacterial activity of *S.hirsuta* and *Indigofera linnaei* with some of bacteria pathogenic strains such as *Shigella dysenteriae, Escherichia coli, Salmonella typhi, Proteus vulgaris, Klebsiella pneumoniae* and *Bacillus subtilis* were experimented. The antibacterial activities of the ethanolic extracts were compared favorably with that standard antibiotic (Chloramphenicol). The Ethanolic extract of leaf showed a maximum zone of inhibition (11 mm) against *Escherichia coli,* a Gram negative bacteria. In *Indigofera linnaei,* the ethanolic extract, show a maximum zone of inhibition (19 mm) to *Salmonella typhi*. In chromatographic technique, the separation and movements of biomolecules has been investigated. Hence, these bio-techniques play a significant role in finding of important material for pharmaceutical industry and have substances that induce a great interest due to their versatile applications. The paper chromatographic technique showed the *Rf* value at chlorophyll 'a' is 0.569 and 'b' value 0.123 present in plant *Crotalaria angulata*. The *Rf* value at chlorophyll'a'0.569, and 'b' value is 0.353 present in the plant *Momordica cymbalaria*. HPLC analyses allow for the identification of samples of *Momordica cymbalaria* with peak value of 1676436 and Retention time is 4.092.This particular study revealed the strong quantitative phytochemicals in *Crotalaria angulata* and *Momordica cymbalaria* and the same has been found to be the most effective free radical quencher. As a culmination, these plant extracts can be a safe alternative to chemical drugs.

Keywords: Phytochemicals, Biomolecules, Evaluation, Chromatographic. Anti-Bacterial

Indian J. Pharm. Biol. Res. (2023): https://doi.org/10.30750/ijpbr.11.1.01

INTRODUCTION

The Phytochemicals are in nature that occurring in the therapeutic plants, leaves, vegetables and roots. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and co mmon sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds [1]. The medicinal values of the plant lies in some organic compounds and the most important of these bioactive constituents are alkaloids, saponins, flavonoids, tannins, glycosides, anthraquinones, steroids and terpenoids [2]. These compounds are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas [3]. The medicinal plants

[1]Department of Botany, St.Xavier's College, Palayamkottai,Tirunelveli, Tamil nadu, India

[2]Department of Botany, Sri Saradha College for Women, Perambalur, Tamilnadu, India

Corresponding Author: Vasanth Nayagam, Department of Botany, St.Xavier's College, Palayamkottai,Tirunelveli, Tamil nadu, India. E-Mail: vasanthbiology@gmail.com

How to cite this article: Nayagam V, Raj JM, Dons T. Evaluation of Phytochemical and HPLC analysis of Crotalaria angulata, Momordica cymbalaria, Senna hirsuta and Indigofera linnaei and it's biological applications. Indian J. Pharm. Biol. Res. 2023;11(1):1-3.

Source of support: Nil

Conflict of interest: None.

Received: 10/01/2023 **Revised:** 22/01/2023 **Accepted:** 08/02/2023 **Published:** 25/03/2023

are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents [4]. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and co mmon sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds [5]. This kind of bio assay would further lead to the process such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the i mmune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property. There are more than thousand known and many unknown phytochemicals. It is well-known that plants produce these chemicals to protect themselves, but recent researches demonstrate that many phytochemicals can also protect human against diseases [6]. The Chemical constituents contain many biologically active compounds that can be extracted from Neem, including alkaloids, flavonoids, triterpenoids, phenolic compounds, Carotenoids, steroids, ketones, triterpenoids and limonoids: saladucin, valassin, meliacin, Nimbin Nimbicin, geducin and Azadirachtin etc..[7-9] .Hence this research paper investigates on two species such as *Crotalaria angulata* and *Mimordica cymbalaria*. And the phyto chemical properties consist in the prescribed plant species. This bioassay would help in the field of biomedical and Pharma industry to get the therapeutic compounds involved. Plants also hold different inorganic nutrients which are essential for growth, development and proper functioning of human body. Ingestion of these inorganic compounds in excess or limited amount can cause various health issues [10-11]. Plant species are believed to contribute to its medicinal properties and this versatile plant have afforded researchers the opportunity to explore its phytochemical diversity [12]. To highlight the importance of these plants, the *S.hirsuta* belongs to Fabaceae family and large herbaceous, Perennial, Seed propagated and Shrub. The herbs are generally regarded as an analgesic with anti-infla mmatory activity. And the second one is *I.linnaei* belongs to family Fabaceae. It is perennial plant with prostrate, it is usually prostrate woody shrub, long taproot system. Because of the presence of bioactive constituents, the selected medicinal plants for this present study have been much focused. Hence the objective of this present study was to perform a phytochemical study and to evaluate the anti-microbial activity of *Crotalaria angulata* , *Momordica cymbalaria, Senna hirsuta* and *Indigofera linnaei* plants.

MATERIALS AND METHODS

Plant identification and sample collection

Crotalaria angulata , *Momordica cymbalaria, Senna hirsuta* and *Indigofera linnaei* were collected locally from Karikudi, Tamil Nadu, India. The taxonomic identification of the plant was carried out at the herbarium of Botany Department and voucher specimens were properly authenticated.

Preparation of plant extracts

Leaves of the plant allowed to air dried in the shade for two weeks and packed in paper bags and stored. Samples were kept under constant observation to avoid any growth of contaminants. Dried leaves were pulverized in an electric grinder to obtain fine power sample. By using this powder different samples were prepared by using organic solvents such as ethanol and distilled water. The samples were stored in a shaker for 72 hours at room temperature. The samples were reduced to 10% of its original volume and filtered using Whatman filter paper. The filtrate was allowed to concentrate in a vacuum by using rotatrory evaporator leaving behind crude extracts.

Phytochemical Screening

Phytochemical analysis of all the evaporated solvent extracts was conducted following the procedure of Indian pharmacopoeia.

Test for Steroids (Salkowski test): Take 2 mL of extract; add 2 mL of chloroform add 2 mL of conc.H_2SO_4 Shake well. Chloroform layer appears red and acid layer shows greenish yellow florescence.

Test for Glycosides (General test): Add 10 mL of 50 % Hcl to the 2 mL of the test solution. Heated and kept in boiling water for 30 min. Add 5 mL of Fehling's solution and boil for 5 min. Brick red precipitate forms.

Test for Anthraquinones Glycosides (Borntrager's test): To 3 mL of the extract, add dil.H_2SO_4. Boil and filter. To the cold filtrate add equal volume of benzene or chloroform. Shake well. Separate organic layer. Add equal volume of dil. A mmonia. A mmoniacal layer turns pink or red.

Test for Saponins Glycosides Foam test: Shake the drug extract or dry powder vigorously with water. Persistent foam forms.

Test for Tannins (Lead acetate test): To 3-5 mL of the test solution, add a few drops of 1% lead acetate. Yellow or red precipitate forms.

Test for Flavonoids (Shinoda test): To the test solution, add 5 mL of alcohol and a few drops of Conc.Hcl and magnesium turnings. Appearance of pink colour.

Test for Terpenoids: To the test solution add 2 mL of chloroform and 1 mL of Conc.H_2SO_4. Appearance of Reddish brown colour.

Test for Saponins (Foam test): To the test solution add a drop of sodium bicarbonate. Gently shake the solution. Honey comb like froth forms.

Bacterial test strains used and growth conditions

The antibacterial activity of *Senna hirsuta* and *Indigofera linnaei* leaves Ethanolic extracts was evaluated using the disc diffusion technique. The test organisms were *Shigella dysenteriae, Escherichia coli, Salmonella typhi, Proteus vulgaris* and *Klebsiella pneumonia.* The bacteria were maintained in Luria Bertani agar media, respectively. All glass wares and other material needed for test were thoroughly sterilized. After 24 hours incubation at 37^{0}C, the diameter (mm) of the inhibition zones was measured.

Anti –microbial activity: Disc diffusion method

Paper discs impregnated with specific antibiotics or the test substances are placed on the surface of the Luria Bertani agar medium inoculated with the target organisms. For the diffusion of antimicrobial agents, the plates are incubated and the zones of inhibition around each disc are measured. Each disc was pressed down to ensure complete contact with the agar surface and distributed evenly. The agar plates were then incubated at 37ºC. After 16 to 18 hours of incubation, each plate was examined. The resulting zones of inhibition were unifor mLy circular with a confluent lawn of growth. The diameters of the zones of complete inhibition were measured, including the diameter of the disc where the Chloramphenicol was used as control.

Chromatography techniques: High-performance liquid chromatography

HPLC analysis was performed on a Shimadzu LC- 10AD VP system equipped with a binary gradient system and SPD-M10A VP photodiode array (PDA) detector. A Hypersil Gold HPLC column (100 mm × 4.6 mm, 3 µm) was used for all experiments. 50 µl of the sample was injected into SIL-10AD VP auto sampler. After injection, the chromatographic conditions were gradually changed through a linear gradient profile to 90% acetonitrile and 10% of the original aqueous mobile phase in 50 min. These conditions were kept stable for 5 min thereafter the column was re-equilibrated to the initial conditions. The flow rate was kept constant at 1 mL/min. The samples for HPLC were prepared by dissolving 3 mg of the dried material in 3 mL of acetonitrile and filtering through a 0.45 µm (Nylon) filter into HPLC vials.

RESULTS AND DISCUSSION

Phytochemical analysis test for *Senna hirsute*

Phytochemical analysis test for *Senna hirsute* showed the phytoconstituents like Steroids, Glycosides, Anthraquinones, Saponins Glycosides and Terpenoids in ethanolic extract (Table 1),

Table 1: Qualitative test for phytoconstituents

S.No	*Experiment*	*Procedure*	*Observation*
1.	Test for Steroids Salkowski test	Take 2 mL of extract; add 2 mL of chloroform and 2 mL of Conc. H2SO4.Shake well.	Appears red and shows greenish yellow florescence.
2.	Test for Glycosides General test	Add 10 mLof 50% HCL to the 2 mL of the test solution. Heat on boiling water bath for 30 min. Add 5 mL of Fehlings's solution and boil for 5min.	Brick red precipitate forms.
3.	Test for Cardiac Glycosides Legal's test	To 3 mL of the extract, add dil.H2SO4.Boil and filter. Add benzene or chloroform. Add equal volume of dil.A mmonia.	A mmonial layer turns pink or red.
4.	Test for Saponins Glycosides Foam test	Shake the drug extract or dry powder vigorously with water.	Persistent foam forms.
5.	Test for Tannins Lead acetate test	To 3-5 mL of the test solution add a few drops of 1% lead acetate.	Yellow or red precipitate forms.
6.	Test for Flavonoids Shinoda test	To the test solution, add 5 mL of alcohol and a few drops of Conc.HCL and magnesium turnings.	Appearance of pink colour
7.	Test for Terpenoides	To the test solution add 2 mL of chloroform and 1 mL of Conc .H2SO4.	Appearance of Reddish brown
8.	Test for Saponins Foam test	To the test solution add a drop of sodium bicarbonate. Gently shake the solution.	Honey comb like froth forms.

but at the same time the flavonoids and Saponins are absent in aqueous extract. Similar results are also showed by [13] where Plants were rich in a wide variety of secondary metabolites such as Steroids, Glycosides, Anthraquinones, Saponins Glycosides and Terpenoids appears biological and pharmacological activities. These may have potential to be used as chemotherapeutic agents or serve as starting material in the developing of new antibiotics. The higher concentrations of more bioactive flavonoids compounds were detected with 70% ethanol due to its higher polarity than pure ethanol. From the present study it is found that crude extract express good biological capacity which indicates that the substance with powerful biological effect exists in this extract and must be isolated and purified to confirm its pharmacological and medical use. The observations and inferences made in phytochemical tests of two medicinal plants such as *Crotalaria angulata* and *Momordica cymbalaria* has been evaluated. These tests show the presence of various bioactive secondary metabolites which is responsible for medicinal attributes. In preliminary qualitative phytochemical analysis of *C. angulata,* plant was done to assessed for the presence of bioactive components (Table 2). The presence of steroids, glycosides, Anthraquinones, saponins glycosides, tannins, flavonoids, terpenoid, and saponins foam was determined. The preliminary qualitative phytochemical analysis of the crude powder of *Momordica cymbalaria* plant was done to assess the presence of bioactive components (Table 3).

Antimicrobial activity for *Senna hirsuta*

The Luria Bertani agar diffusion method was used to evaluate the antimicrobial activity by measuring the zone of inhibition against the test microorganisms. The Ethanolic and aqueous extracts of *Senna hirsuta* show antimicrobial properties against wide spectrum of bacteria (two gram-positive and four gram-negative species). The antibacterial activities of the ethanol extracts compared favourably with that standard antibiotic (Chloramphenicol). The ethanolic extract of tested plant shows a varying degree for anti-bacterial activity. The Ethanolic extract of leaf showed a maximum zone of inhibition (11 mm) against which is *Escherichia coli* Gram negative bacteria (Table 5). People and animals normally have some *E. coli* in their intestines, but some strains cause infection. The bacteria that cause infection can enter into your body in a number of ways. Practicing safe food behaviours can decrease your chances of developing an intestinal infection due to *E. coli.*

Phytochemical analysis test for *Indigofera linnaei*

The phytochemical analysis test for the highly significant amount of steroids, glycosides, saponins glycosides and terpenoids are present in ethanolic extract, but at the same time the anthraquinones, tannins, flavonoids and saponins are absent in aqueous extract (Table 6 and Figure 1).

Table 2: The details of phytochemical analysis of *C. angulata*

S. no	*Experiment*	*Ethanol*	*Water*
1.	Test for steroids	+	+
2.	Test for glycosides	_	+
3.	Test for anthraquinones	+	_
4.	Test for saponins glycosides foam	_	_
5.	Test for tannins lead acetate test	+	+
6.	Test for flavonoids	_	_
7.	Test for terpenoids	+	+
8.	Test for saponins foam test	_	+

Table 3: The details of phytochemical analysis of *M.cymbalaria*

S.no	Experiment	Ethanol	Water
1.	Test for Steroids	+	+
2.	Test for Glycosides	+	+
3.	Test for Anthraquinones	_	_
4.	Test for Saponins glycosides	_	_
5.	Test for Tannin	+	+
6.	Test for Flavonoids	_	_
7.	Test for Terpenoids	+	+
8.	Test for Saponins Foam test	+	+

(+) Indicate the presence of phytochemicals and
(-) indicate the absence of phytochemicals.

Table 4: The details of Phytochemical analysis of *S. hirsuta*

S. no.	*Variable*	*Ethanol*	*Water*
1	Steroids	+	+
2	Glycosides	+	+
3	Anthraquinones	+	+
4	Saponins glycosides	+	+
5	Tannins	_	+
6	Flavonoids	+	_
7	Terpenoids	+	+
8	Saponins	_	_

Antimicrobial activity for *Indigofera linnaei*

The ethanolic and aqueous extracts of *Indigofera linnaei* tested showed varying degree of antibacterial activities against the test bacterial species. Antibacterial activities of extracts were evaluated by measuring the zone of inhibition. The antibacterial activities of the ethanol extracts compared

Table 5: Anti-Bacterial activity of *S.hirsuta*

S. no	*Bacterial names*	*Ethanolic extract*	*Chloramphenicol*	*Aqueous*
1	*Shigella dysenteriae*	9.66 ± 0.47	15.33 ± 2.05	7.66 ± 0.47
2	*Escherichia coli*	11.33 ± 0.47	16.33 ± 0.47	8.33 ± 1.24
3	*Salmonella typhi*	11 ± 0.81	14.66 ± 1.24	9.66 ± 0.47
4	*Proteus vulgaris*	10.66 ± 0.47	14.66 ± 1.24	8 ± 1.41
5	*Klebsiella pneumoniae*	10.66 ± 0.47	13.33 ± 0.47	8.33 ± 1.69

All values are expressed as mean ± S.E.M

Table 6: The details of Phytochemical analysis of *I. linnaei*

S. no.	*Variable*	*Ethanol*	*Water*
1.	Steroids	+	+
2.	Glycosides	+	+
3.	Anthraquinones	_	+
4.	Saponins Glycosides	+	+
5.	Tannins	_	_
6.	Flavonoids	_	_
7.	Terpenoids	+	_
8.	Saponins	_	+

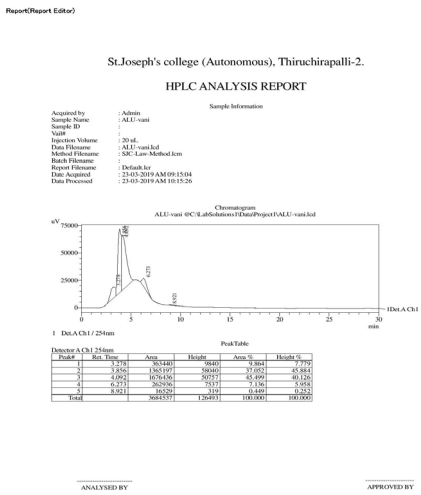


Figure 1: HPLC analysis of *Momordica cymbalaria*

favourably with that standard antibiotics (Chloramphenicol) and have appeared to be broad spectrum as its activities were independent on gram reaction. The ethanol extract leaves exhibit strong antimicrobial activity against all the tested organisms. The ethanolic extract of leaf showed a maximum zone of inhibition (19 mm) against which is a *Salmonella typhi* Gram negative bacterium (Table 7 and Figure 2).

High performance liquid chromatography (HPLC)

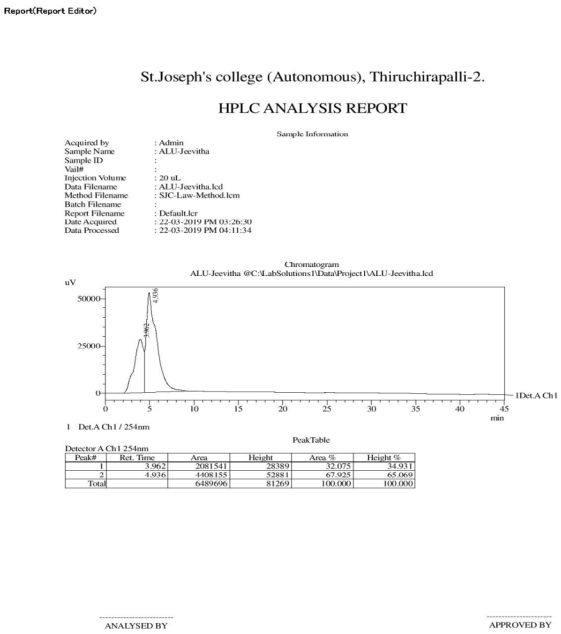
HPLC is an analytical technique for the separation and determination of organic and inorganic solutes in any samples especially biological, pharmaceutical, food, environmental, industrial etc., qualitative and quantitative analysis. The another name for HPLC is High Pressure Liquid Chromatography, separates compounds on the basis of their interactions with solid particles of tightly packed column and the solvent of the mobile phase. Modern HPLC uses a non-polar solid phase, like C18 and a polar liquid phase, generally a mixture of water and another solvent. The HPLC analysis of *Senna hirsute* (Figure 3) ethanolic extract revealed significant levels of chemical compounds and are shown. The compounds obtained in the chromatogram the retention times of 3.962 the peak area 32.075, height 34.931. The retention times of 4.936 the peak area 67.925, height 65.924 were close to the reference compounds.

Paper Chromatography: The distance travelled by *Rf* value of chlorophyll 'a'= 0.569 and The chromatography distance travelled by *Rf* value of chlorophyll 'b'= 0.123. *Momordica cymbalaria* The chromatography distance travelled by *Rf* value of chlorophyll 'a'= 0.569 The chromatography distance travelled by *Rf* value of

Table 7: Anti-Bacterial activity of *I. linnaei*

S. no	*Bacterial names*	*Ethanolic extract*	*Chloramphenicol*	*Aqueous*
1.	*Salmonella typhi*	19.66 ± 4.49	17 ± 0.81	10.33 ± 0.47
2.	*Shigella dysenteriae*	13 ± 1.63	15.66 ± 0.47	9.66 ± 0.47
3.	*Klebsiella pneumoniae*	9.66 ± 0.47	17.66 ± 1.69	7.33 ± 0.47
4.	*Bacillus subtilis*	11 ± 0.81	12.66 ± 3.68	8.66 ± 1.88
5.	*Escherichia coli*	11.33 ± 0.47	16.33 ± 0.47	9.66 ± 0.52

All values are expressed as mean ± S.E.M



Report(Report Editor)

St.Joseph's college (Autonomous), Thiruchirapalli-2.

HPLC ANALYSIS REPORT

Sample Information

Acquired by	: Admin
Sample Name	: ALU-Jeevitha
Sample ID	:
Vial#	:
Injection Volume	: 20 uL
Data Filename	: ALU-Jeevitha.lcd
Method Filename	: SJC-Law-Method.lcm
Batch Filename	:
Report Filename	: Default.lcr
Date Acquired	: 22-03-2019 PM 03:26:30
Data Processed	: 22-03-2019 PM 04:11:34

Chromatogram

ALU-Jeevitha @C:\LabSolutions\Data\Project1\ALU-Jeevitha.lcd

1 Det.A Ch1 / 254nm

PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.962	2081541	28389	32.075	34.931
2	4.936	4408155	52881	67.925	65.069
Total		6489696	81269	100.000	100.000

ANALYSED BY

APPROVED BY

Figure 2: HPLC analysis of *Senna hirsuta* ethanolic extract

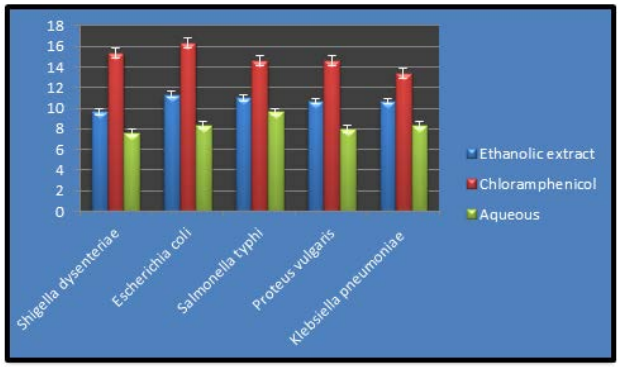


Figure 3: Anti-Bacterial activity of *S.hirsuta*

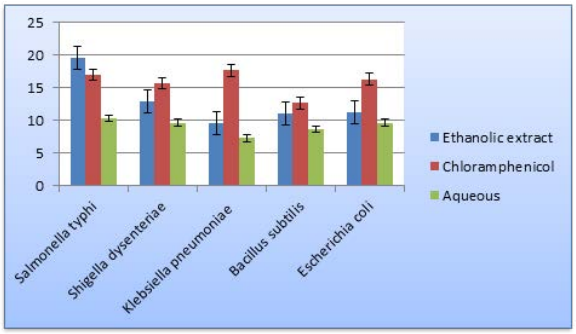


Figure 4: Anti-Bacterial activity of *I. linnaei*

chlorophyll 'b'= 0.353. HPLC is a versatile, robust, and widely used technique for the isolation of natural products. In *Crotalaria angulata* and *Momordica cymbalaria* the Retention time is time interval between sample injection and the maximum of the peak. The Peak value present in Area of 1676436 and Retention time for 4.092. As shown in the (Figure 4) the next peak value for Area is 1365197 and their Retention time for 3.856. The next peak value for Area is 363440, and Retention time for 3.278.

CONCLUSION

Therefore, increase in failure of chemotherapeutics and antibiotic resistance leads to screening of several medicinal plants for their antimicrobial effect. Hence, this present would help the Pharma industry and medical industry where the drug designing may play a vital role and same would benefit more. With this background, in the present study an attempt to make systematically evaluate phyto chemical and anti-microbial property of these two medicinal plants. The presence of most general phytochemicals might be responsible for their therapeutic effects. It is further reflects a hope for the development of many more novel chemotherapeutic agents or templates from such plants which in further may serve for the production of synthetically improved therapeutic agents. Curing diseases using plants was practiced from ancient and primitive times and continued even now among the tribal's in various countries. It is widely confirmed that curative property of medicinal plant is mainly related to the presence of secondary metabolites in the plants. Phytochemicals are chemical compounds produced by, plants generally to help them thrive or thwart competitors, predators, or pathogens. Hence in this present study, we have investigated the chemical composition of aromas and essential compounds. Phytochemical screening of these two plants has revealed the presence of various bioactive phytocompounds.

ACKNOWLEDGEMENTS

The Authors would like to thank Alagappa University towards the completion of this research work and publication.

CONFLICT OF INTEREST

The authors state no conflict of interest.

Research Involving Human Participants and/or Animals

This article does not contain any studies with human participants or animals performed by any of the authors.

REFERENCES

1. Krishnaiah D, Sarbatly R and Bono A. Phytochemical antioxidants for health and medicine: A move towards nature. *Biotechnol Mol Biol Rev.* 2007; 1:97-104.
2. Dhandapani R and Sabna B. Phytochemical constituents of some Indian medicinal plants. *Ancient Science of Life.* 2008; 37(4): 1-7.
3. Vasu K, Goud J, Suryam V, Singara A and Chary M. Biomolecular and phytochemical analyses of three aquatic angiosperms. *African Journal Of Microbiology Research.* 2009; 3(8): 418-421.
4. Nostro A, Germanò MP, D'angelo V, Marino A, Cannatelli MA. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Lett Appl Microbiol.* 2000; 30: 379-384.
5. Krishnaiah D, Sarbatly R, Bono A. Phytochemical antioxidants for health and medicine: A move towards nature. *Biotechnol Mol Biol Rev.* 2007; 1: 97-104.
6. Rao N. Bioactive phytochemicals in Indian foods and their potential in health promotion and disease prevention. *Asia Pacific Journal of Clinical Nutrition.* 2003; 12 (1): 9-22.
7. Lale, NES, Bio-activity and Limitation against wide spread use of neem products for the management of insect pests. *Nigerian J. Applied Biol* .2002; 3:115–125.
8. Verkerk R.H.J, Wright D.J. Biological activity of neem seed kernel extract and synthetic azadirachtin against larvae of *Plutella xylostellal*. *Pesticide science.* 1993; 37: 83-91.
9. Singh V and Chauhan D. Phytochemical evaluation of aqueous and ethanolic extract of neem leaves (*Azadirachta indica*). *Indo American Journal of Pharmaceutical Research* .2014; 4(12).
10. Arvind K S. Medicinal plants: Future source of new drugs. International Journal of Herbal Medicine. 2016; 4(4): 59-64.
11. Joya mma V, Seema S, Nair. Phytochemical, Physico Chemical and Elemental Analysis of Leaves and Stem of *Pothos scandens* Linn. International Journal of Pharmacognosy and Phytochemical Research .2019; 11(2):37-43.
12. Kevin A, Reaa, José A. Casarettoa M, Sameer Al-Abdul-Wahidb, Arjun S, Jennifer G, Steven J, Rothsteina, Tariq A, Akhtara. Biosynthesis of cannflavins A and B from Cannabis sativa L. Phytochemistry. 2019;(164): 162–171.
13. Okwu DE and Josiah C. Evaluation of the chemical composition of two Nigerian medicinal plants. *Afr J Biotec* 2006; 5: 357-361.