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Evaluation of Antimicrobial and Anti-Inflammatory properties in *Quisqualis indica* L., *Mimosa pudica* L. and *Antigonon leptopus* Hook &Arn.

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ABSTRACT
The nature is a source of various auspicious things for human, one of them is the therapeutic plants. These therapeutic plants are source of various important compounds which may be found in their roots, stems, leaves or flower. These effective compounds are known as the bioactive compounds or the phytochemical compounds. These phytochemical compounds are potent of carrying out various effective biological properties like antimicrobial, antioxidant, anticancerous, anti-inflammatory etc. The present study was carried out on three therapeutic plants including Q. indica L., M. pudica L..and A. leptopus Hook & Arn. These plants were taken under consideration to determine the presence of bioactive compounds possessing the anti-inflammatory properties. The dried leaf samples of the selected plants were used to prepare extract in solvents. The extract was analysed for the presence of different phytochemical compounds and they were also characterized by UV-visible spectra and HPLC. The extracts were undertaken for their biological activity including the antibacterial, anti-fungal and antioxidant activity. The results obtained from the study depict that all the three plant are good source of the potent bioactive compounds like alkaloids, flavonoids and glycosides. The result of biological activity analysis demonstrates that the extracts are capable of carrying out the reasonable activity. The HPLC analysis showed the presence of bioactive compound quercitin (flavonoid) in the extracts, which depict that the extracts may possess a good anti-inflammatory property too.
Keywords: Medicinal plants, anti-inflammatory, twiners, phytochemicals, spectra, chromatogram, flavonoids

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INTRODUCTION

The therapeutic plants which are also popularly known as herbal medicines, phytomedicines or botanical medicines constitute majorly of roots from plants, their stems, leaves, seeds and other natural parts of plants which could be used for betterment of health of living beings. These traditional herbal products are either used in their natural state or transformed into some readymade products in used day to day life**.** The phytochemical compounds of the plants are the active constituents of medicinal plants which impart the advantageous properties to them thus making useful for humans. The recognition to these properties has been given by people since ancient times. The knowledge on these properties have come through many generations especially in countries of Africa including Cameroon, Mali, Nigeria and Zambia [18, 21].

Quisqualis indica L. (Family - Combretaceae, Bengali name - Modhumaloti) is an evergreen creeping shrub, which can be as much as 70 feet long in tropical climates [38]. The leaves of *Q. indica* L. are opposite and 7-15 cm long. Its flowers have a faint sweet aroma and they bloom in the spring, early summer or mid fall [38]. Two diseases for which their fruit are used in treatment are ascariasis and oxyuriasis [5]**,** while the decoction of the fruit is useful in toothache and nephritis. The roasted ripe seeds are beneficial in diarrhea, fever and rickets. Furthermore, seeds macerated in oil can also be applied in parasitic skin troubles [11]**.** Decoction of leaves is useful in abdominal pain and the leaf juice is a remedy for boils and ulcers. The leavesare also used to treat various kinds of infantile disorders and skin diseases, whereas the rootsare used in rheumatism and diarrhea. Besides, the plant extract has been found to be anticoccidial for veterinary purposes [39]**.** Previous phytochemical studies with the *Q. indica* L. led to the

isolation of quisquagenin, quisqualic acid, quisqualin A and quisqualin B [12]. In this paper, we report the preliminary antimicrobial activity and cytotoxicity of *Q. indica*L. extractives for the first time.
Mimosa pudica L. is a creeping annual or perennial herb. It has been identified as lajjalu in Ayurveda and has been found to have antiasthmatic, aphrodisiac, analgesic, and antidepressant properties. *M. pudica* L. is known to possess sedative, emetic, and tonic properties, and has been used traditionally in the treatment of various ailments including alopecia, diarrhea, dysentery, insomnia, tumor, and various urogenital infections. The Phytochemicals which are reported in *M. pudica* L. are alkaloids, non-protein amino acid (mimosine), flavonoids C-glycosides, sterols, terpenoids, tannins, and fatty acids [15]**.** Two well-known movements are observed in *M. pudica* L. (ojigi-so in Japanese): one is the very rapid movement of the leaves when it is stimulated by touch, heating, etc., and the other is the very slow, periodical movement of the leaves called nyctinastic movement which is controlled by a biological clock [36]**.** The leaves of the sensitive plant *M. pudica* can adapt their closing response to electrical and mechanical stimulation so that they reopen to repeated stimulation. The more intense the stimuli and the longer the intertribal interval, the longer it takes to adapt. Leaves adapted to the effects of mechanical stimulation can still respond by closing to electrical stimulation and vice versa [2].
Antigonon leptopus Hook & Arn. belongs to family Polygonaceae and it is commonly found in tropical Asia especially in China and India, Africa, the Caribbean and the Americas [34]. *A. leptopus* plant flowers are also used in omelets [8]. A hot tea prepared from the aerial portion of this plant, is used as a treatment for cough and throat constriction in Jamaica and considered as one of the important medicinal plants in their folk-medicine [27, 28, 37]. Previous studies have shown that *A. leptopus* plant extracts, exhibited potential anti-thrombin and anti-diabetic activities [10, 22, 3] and reported its anthelmintic activities properties [35].
Inflammation forms a part of the complex biological response of our vascular tissue by harmful stimuli such as pathogen or damaged cells**.**Plants possess property to synthesize phytochemical compounds which can show inhibitory effect against many fatal diseases. Anti-inflammatory effects of such herbal plants are under scan since a long period. The aim of this study was to evaluate three plants *Quisqualis indica*L.*, Mimosa pudica*L.and *Antigonon leptopus*Hook & Arn. for their antibacterial, antifungal and anti-inflammatory action. An insight into these properties would help in better understanding of chemical constituents of these plants and thus help in boosting their role for health benefits of human.

MATERIAL AND METHODS

Chemicals used

The present study was carried out using chemicals purchased from Hi-Media, India. The chemicals and reagents used were of Analytical grade.

Plant material collection and their identification

The plant samples *Quisqualis indica, Mimosa pudica*and *Antigonon leptopus* were collected fromdifferent areas of West Champaran, Bihar (India). The samples were first processed by washing them thoroughly under running tap water and then dried in shade for 7 days at room temperature. After complete removal of moisture from plants, they were ground to powder usingelectric grinder and stored in air tight pouches for further analysis.

Extract preparation

The three powdered plant samples of *Quisqualis indica*L*, Mimosa pudica* L.and *Antigonon leptopus* Hook & Arn. were extracted separately with Methanol and toluene using Soxhlet apparatus80°C-3 cycles for 12 h. 10g plant material were dissolved in 100ml of solvent and extracted successively with toluene and methanol. The plant extracts were dried completely and then redissolved in respective solvents (10mg extract in 10ml of solvent). These extracts were then used for preliminary phytochemical screening, antibacterial, antifungal and antioxidant analysis.

Qualitative analysis of phytochemicals

Preliminary screenings of the phytochemicals compounds in each extract were analyzed qualitatively following standard method described by Jamil *et al.* [20]. Majorly four constituents were screened which included alkaloids, glycosides, flavonoids and phenolic compounds.

UV-Visible spectrum of the plant extracts

The UV-visible spectra for the plant extracts were taken by using UV-visible double beam spectrophotometer. The range of spectra was 200nm-800nm. The extracts were placed in the cuvette against the solvent (blank- methanol/toluene). The spectra were measured using the software of the

instrument. The result obtained revealed the presence of phenols and flavonoids in the extract as the peak were prominent in the range of 300-400nm.

Antibacterial and Antifungal Activity

The antibacterial activity of extracts of *Quisqualis indica* L, *Mimosa pudica* L. and *Antigonon leptopus* Hook & Arn. were assessed against two bacterial strains, one gram positive *Staphylococcus aureus*, one gram negative *Pseudomonas aeruginosa* and one fungus *C. albicans* using the agar well diffusion method as reported earlier [20]. Plant extracts (20 µl), three positive controls (500ppm ciprofloxacin for *P. aeruginosa*; 500ppm Norfloxacin for *S. aureus*; 1000ppm fluconazole for *C. albicans*)) and negative control (DMSO) were used on each plate.

Antioxidant activity(DPPH (2,2-diphenyl1-1-picryl-hydrazyl radical) free radical scavenging) of the plant extract

The antioxidant activity was assessed for the methanolic and toluene extracts of *Quisqualis indica* L, *Mimosa pudica* L. and *Antigonon leptopus* Hook & Arn. The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging activity of the six test extracts was evaluated as previously explained [13].The plant extracts were taken in different concentrations and analyzed for the test. The decrease in the absorbance at 517 nm was taken as the antioxidant capacity of the sample. 150µl of DPPH solution was mixed in 3ml methanol and absorbance was taken immediately at 517nm for control reading. The absorbance for control was 0.193. Methanol was used as blank. The antioxidant activity was calculated in the form of % scavenging activity using the formula and a graph was plotted from it; Percent (%) inhibition or scavenging= [(absorbance of control-absorbance of test sample)/absorbance of control] * 100.

HPLC Analysis

The methanolic and toluene extracts of *Quisqualis indica* L, *Mimosa pudica*L. and *Antigonon leptopus* Hook & Arn. were subjected to HPLC analysis of the instrument HPLC (Systronics, India) with HiQ Sil C18-HS column (column size:4.6 mm × 250 mm × 5µM; 25°C), isocratic pump and injecting loop of sample 20 µl. Methanol was used as solvent system for elution of the sample with standard (quercitin) 10 µg/ml (dissolved in methanol). The stock solution of concentration 10 µg/ml was prepared by dissolving 10µgstandard in 0.5 ml HPLC-grade methanol followed by sonication for 10 minutes and the resulting volume was made up to 1ml with the methanol. The standard and sample solutions were filtered through 0.22µm PVDF-syringe filter and the mobile phase was degassed before the injection of the solutions. HPLC chromatograms were detected using a photodiode array UV detector at single wavelengths of 254nm according to absorption maxima of analysed compounds. Each compound was identified by its retention time and by spiking with standards under the same conditions. The quantification of the sample was done by the measurement of the integrated peak.

RESULT AND DISCUSSION

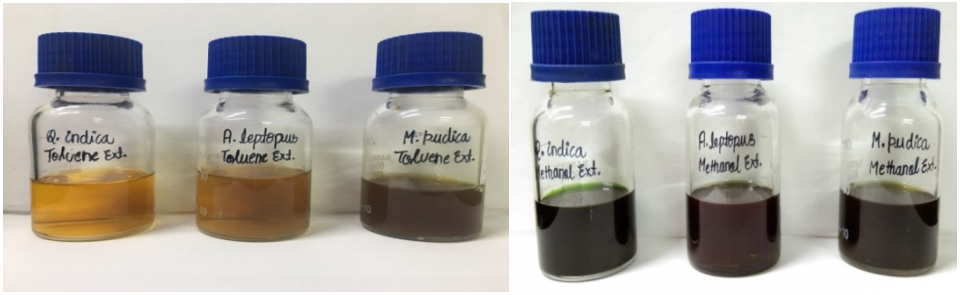


Figure 1: The extracts of respective plant in methanol and Toluene solvent

Phytochemical analysis:

The extracts obtained were undertaken for the analysis of their phytochemical compounds. The phytochemical tests were carried out for the detection bioactive compounds like Phenol, Glycosides, Flavonoids and Alkaloids in the each plant extract. The test was carried out for both solvents of each plant. The test result showed the presence of majorly Phenol and Glycosides in the plant extract while some extract showed the presence of alkaloids and flavonoids in them. The result obtained by the phytochemical analysis of the plant extracts is summarized in the table no. 1 given below.

Table 1: The summarized result of the phytochemical analysis of the plant extract for both solvents

S. No.	Phytochemical test	*Q. indica* L.		*A. leptopus* Hook & Arn.		*M. pudica*L.	
		Methanol	Toluene	Methanol	Toluene	Methanol	Toluene
1	Phenol test	+	+	+	-	+	+
2	Glycosides test	+	+	+	-	+	+
3	Flavonoid test	+	-	+	+	-	+
4	Alkaloid test	+	+	-	-	+	-

(+)- Present (-)-Absent

UV-Visible Spectrum of the plant extracts:

The UV-visible spectra for the plant extracts were taken by using UV-visible double beam spectrophotometer. The range of spectra was 200nm-800nm. The sample was placed in the cuvette against the blank containing the solvent of plant extract that is methanol and toluene. The spectra were measured using the UV-Visible double beam spectrophotometer software of the instrument. The result obtained revealed the dominating presence of phenols and flavonoids in the extract as the peak were prominent in the range of 300-400nm as shown in the figure 2 (*Q. indica*); figure 3 (*A. leptopus*) and figure 4(*M. pudica*).

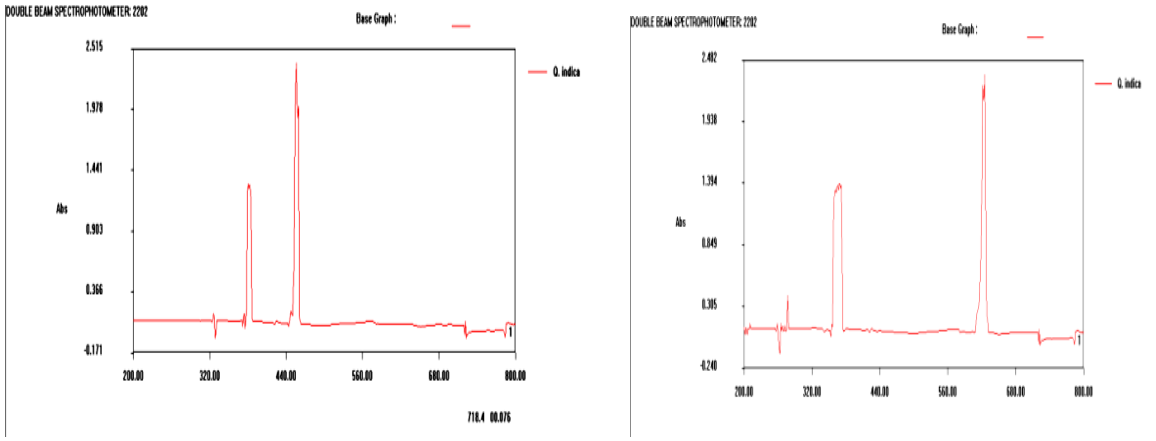


Figure 2: Showing result of UV-Visible spectra for Q. indica (Methanol extract and Toluene extract)

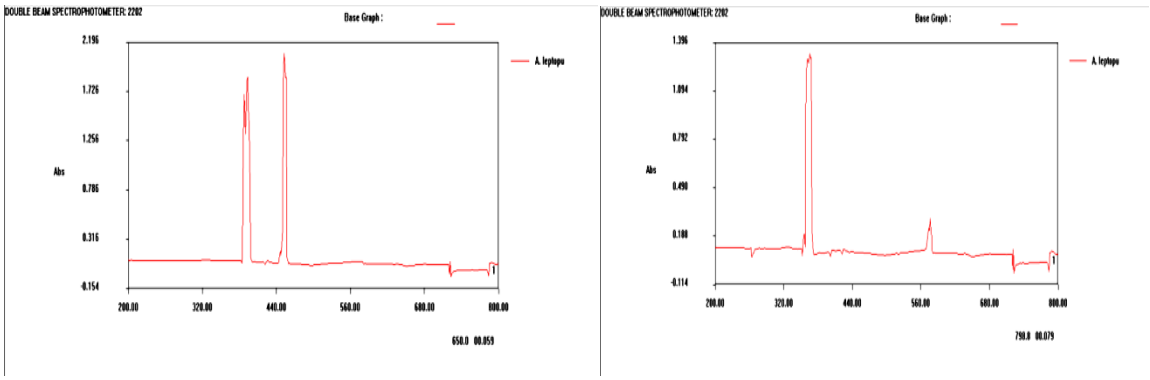


Figure 3: Showing result of UV-Visible spectra for *A. leptopus Hook* & Arn. (Methanol extract and Toluene extract)

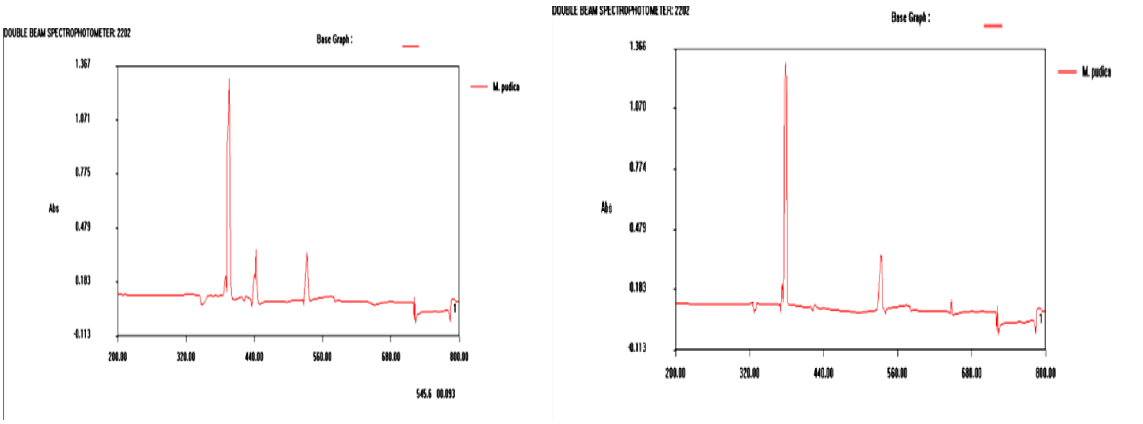
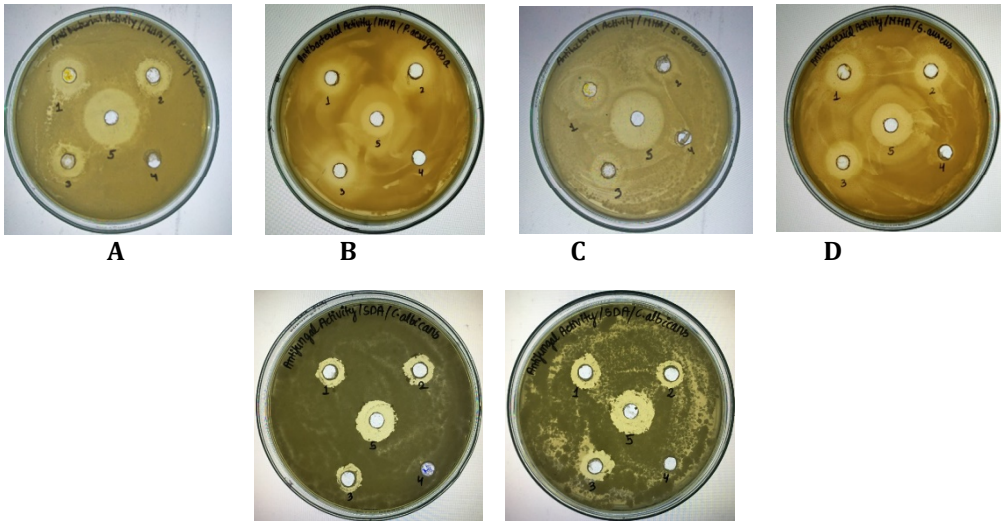


Figure 4: Showing result of UV-Visible spectra for *M. pudica* L. (Methanol extract and Toluene extract)

Antimicrobial activity of the plant extracts:

The Antimicrobial activity of the plant extract was determined by Agar well diffusion method. The extracts (20µl) were loaded in specific well punctured on the inoculated medium. The media was inoculated with the bacterial strain of *Pseudomonas aeruginosa* and *Staphylococcus aureus* and the fungal strain used was *Candida albicans*. The positive controls were loaded in the same quantity in the centre well. The positive control used was 1000ppm oxy-tetracycline antibiotic for bacteria and for fungi it was 10000 ppm of fluconazole antibiotic. The result was analyzed on the basis of the Zone of Inhibition (measured in mm) obtained around the well. The result of antimicrobial analysis is summarized in the table below.



A **B** **C** **D**

E **F**

Figure 5: Showing result of Antimicrobial activity of Plant extract against *P. aeruginosa (A-methanolic* extract*;* B-toluene extract); *S. aureus* (C-methanolic extract; D-toluene extract); *C. albicans* (E-methanolic extract; F-toluene extract)

In the picture above; 1- *Q. indica* L., extract; 2- *A. leptopus* Hook & Arn., 3- *M. pudica*L; 4-negative control; 5-positive control (500ppm ciprofloxacin for *P. aeruginosa*; 500ppm Norfloxacin for *S. aureus*; 1000ppm fluconazole for *C. albicans*).

Table 2:The result for the Antimicrobial assay of the plant extracts

Microbe	Zone of Inhibition (mm)						
	***Q. indica* L.**		***A. leptopus* Hook & Arn.**		***M. pudica*L.**		**Positive control**
	Methanol	**Toluene**	**Methanol**	**Toluene**	**Methanol**	**Toluene**	
P. aeruginosa	18	15	16	15	16	14	20
S. aureus	18	18	13	15	15	17	20
C. albicans	14	15	13	11	13	12	21

Antioxidant activity assay of the plant extract:

The antioxidant activity of the plant extracts was checked by DPPH (2, 2-diphenyl-1- picrylhydrazyl) radical scavenging activity. The plant extracts were taken in different concentrations and analyzed for the test. The decrease in the absorbance at 517 nm was taken as the antioxidant capacity of the sample. 150ul of DPPH solution was mixed in 3ml methanol and absorbance was taken immediately at 517nm for control reading. The absorbance for control was 0.193. Methanol was used as blank. The antioxidant activity was calculated in the form of % scavenging activity using the formula and a graph was plotted from it; Percent (%) inhibition or scavenging = [(absorbance of control-absorbance of test sample)/absorbance of control] * 100.

The results depicted that the plant extracts possess a good level of antioxidant activity in a concentration dependent manner. The IC_{50} value for each plant extract's antioxidant was calculated and the least value was found with the methanolic extract of *Q. indica* L., indicating the best antioxidant property among all the extracts.Value for IC_{50} for each extract was calculated from the graph placing the value of y=50 and calculating for the value of x.The value of %scavenging activity for each extract at every concentration and the IC_{50} value for each extract is depicted in the tables below.

Table 3:table for the % scavenging activity and $IC_{50}$for different concentration of all the plant extracts

Concentration (µg/ml)	Scavenging activity					
	Extract 1	Extract 2	Extract 3	Extract 4	Extract 5	Extract 6
100	26.4248705	24.8704663	31.0880829	27.97927461	25.38860104	23.31606218
200	35.2331606	34.1968912	40.41450777	35.75129534	28.49740933	26.42487047
300	50.7772021	50.7772021	48.70466321	41.96891192	40.41450777	37.30569948
400	57.5129534	56.9948187	52.33160622	49.74093264	49.74093264	47.15025907
500	63.7305699	62.6943005	56.99481865	53.88601036	53.36787565	51.29533679
IC_{50}	333.66µg	341.73 µg	364.39 µg	423.64 µg	436.25 µg	468.14 µg

Extract 1: Methanolic extract of *Q. indica*; L. Extract 2: Toluene extract of *Q. indica* L.
Extract 3: Methanolic extract of *A. leptopus* Hook & Arn. Extract 4:Toluene extract of *A. leptopus* Hook & Arn.
Extract 5: Methanolic extract of *M. pudica* L; Extract 6: Toluene extract of *M. pudica* L.

Detection of Phytochemicals by HPLC:

The plant extracts were analyzed for the presence of bioactive compound by HPLC. The analysis was done for all the plant extracts. The result of analysis revealed the presence of bioactive compound quercitin (flavonoid) in the plant extracts. The presence of quercitin in extracts was confirmed by HPLC analysis of quercitin standard. The peak position and retention time of quercitin standard and sample extracts overlaps indicating the active presence of quercitin in the plant extracts.

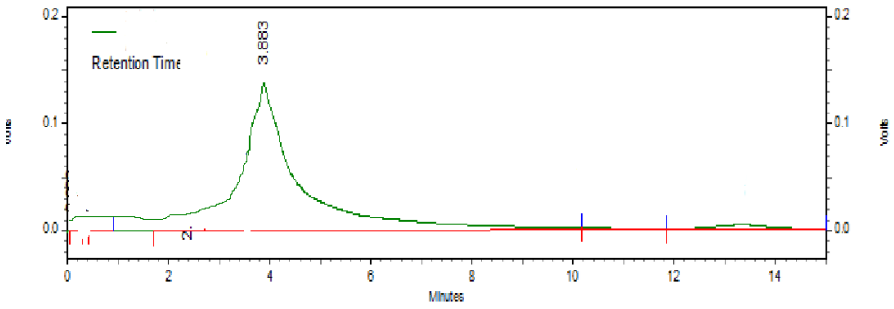


Figure 6: HPLC chromatogram of standard quercitin

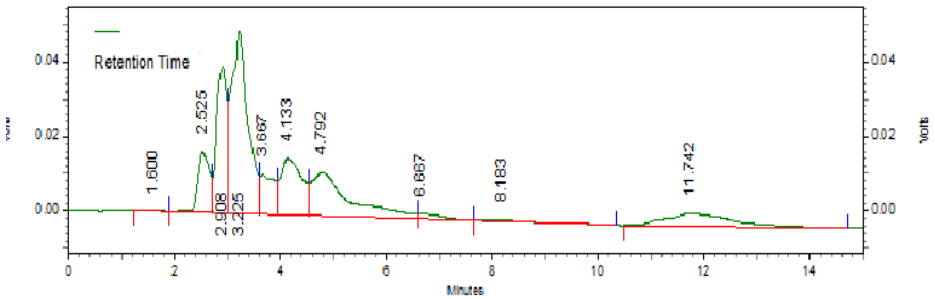


Figure 7: HPLC chromatogram of methanolic extract of *Q. indica* L.

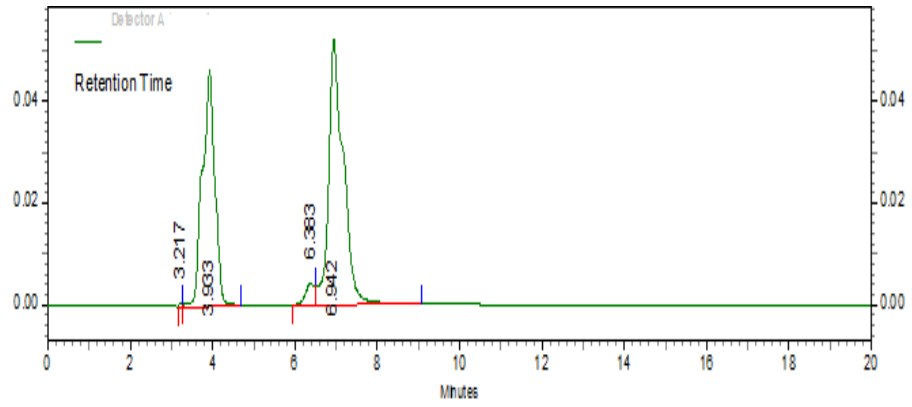


Figure 8: HPLC chromatogram of methanolic extract of *A. leptopus* Hook & Arn.

DISCUSSION

Ayurveda and traditional medicines holds several records of treating the people with phytochemical compounds suffering from inflammation and pain. The inflammatory responses occur in three distinct phase *i.e.* acute phase, sub-acute phase, and chronic phase. There are several records available on the plants in traditional medication or ethno-medicines that focused on the relief of pain, swelling, fever, inflammation and rheumatism [26].

The antioxidant potential of plants is directly related with their therapeutic potential [13]**.** The plant extracts showed a good range of antioxidant properties hence indicating their potential to be used as therapeutic agents. The qualitative phytochemical screening of the plants extracts have revealed the presence of several potent bioactive compounds in the plant extracts which include phenolic compounds, alkaloids, glycosides and flavonoids. The presence of such potent bioactive compounds in the extract is responsible for their anti-inflammatory effects. The results of phytochemical screening of all the plant extracts is compared with the previous findings like, the methanolic extract of *M. pudica* L. showed the presence of alkaloids, phenols and glycosides as reported by [30]**;** extract of *Q. indica* L. showed the presence of alkaloids, flavonoids, phenols and glycosides as reported by the findings of [19]; phytochemical screening of *A. leptopus* Hook & Arn. was similar with the findings [14].

As reported by Ahmad *et al*, who found that flavonoids and tannins possess a good anti-inflammatory effects [1]. The presence of flavonoid in the plant extracts undertaken in the study reveals their potential to act as an anti-inflammatory agent. Flavonoid like Quercetin is shown to be useful for treating acute inflammation [33]. The presence of Quercetin in the plant extracts showing flavonoid positive is confirmed via the HPLC analysis. Quercetin has a large spectrum of biological activities like anti-inflammatory [6], anti-infectious [9], antioxidant [7] and anti-hypersensitive [23]. Quercetin is known to target the pro-inflammatory signaling pathways such as STAT1, NF5B and MAOK [16, 24]. These studies hence reveal that the presence of flavonoid (Quercetin) in the plant extracts make them potent to be used as anti-inflammatory agents. The mode of action of flavonoids includes by inhibiting the key enzymes responsible for synthesizing prostaglandins [25] and by inhibiting several other enzymes like phosphodiesterase, phospholipase A2, tyrosine kinase and protein kinase [27]. The Flavonoids are known to inhibit the inflammation by inhibiting the signal transducer and activator of transcription 1 (STAT-1) and nuclear factor kappa beta (NF- kβ) activation [16]**.**

The extract of medicinal plants possesses a good antibacterial effect as well. The studies have shown that the presence of some bioactive compounds is responsible for their property. The presence of flavonoids and phenolic compounds in plant extract reveal their potency to act as antibacterial agent. The Flavonoids like robinetin, myricetin and epigallocatechin inhibits the growth of *Proteus vulgaris* and *Staphylococcus aureus* [31] while other study indicated that kaempferol, myricetin, naringin, quericitin and rutin contributes the inhibition against microbes like *P. macrocarpa* [17]. The mechanism of action of the phytochemical compounds being antimicrobial in nature includes the inhibition of peptidoglycan synthesis, destruction of microbial membrane structures and modification of hydrophobicity of the bacterial membrane.

The phytochemical screening has also revealed the presence of alkaloids in some plants extracts. The presence of alkaloid in plant extracts was also shown by UV-visible spectrum. A wide range of biological effects has been reported for alkaloids, including emetic, anti-cholinergic, antitumor, diuretic, sympatho-mimetic, antiviral, antihypertensive, hypno-analgesic, antidepressant, mio-relaxant, anti-tussigen, antimicrobial and anti-inflammatory activities [2].The Alkaloids are found to reduce the intensity of

oedema (watery fluid collecting in the cavities or tissues of the body) by inhibiting with the vascular permeability that is induced by action of histamine [32].

These findings and the discussion over the important biological activities of the bioactive compounds of plant extract has revealed that the plant extracts containing the potent phytochemicals compounds can be administered as the effective measure to treat several disorders. The discussion also reveals the importance of these bioactive compound for the treatment of inflammation hence the incorporation of such plant extracts possessing the anti-inflammatory phytochemicals could improve the course of treatment of inflammation as due to their natural origin they confer less or no side effects on the individuals contrast to this the NSAIDS used for the treatment of inflammation cause some side effects on individual and in prolonged use may lead to severe damage as well.

CONCLUSION

The present study considered three plants, *Q. indica* L.*, A. leptopus* Hook & Arn. *, M. pudica* L. and derived their phytochemical screening. The result of phytochemical screening revealed the presence of potent bioactive compounds in all the three plants. The plant extracts also showed a promising antioxidant property hence emphasizing on their medicinal importance. The major bioactive compounds found to be present the extracts were flavonoids, phenols, alkaloids and glycosides.

The main aim of study was to detect the presence of the bioactive with potent anti-inflammatory properties. As the discussion reveals the bioactive compounds found in the plant extracts have considerable and good anti-inflammatory properties. Their presence makes the plant as an important medicinal source. Since all the three plants considered in the study contains the effective bioactive compounds with not only anti-inflammatory but other biological effects too, these plant needs to be used for the preparation of herbal medicines for treating the inflammation reaction. The bioactive compound not only controls the inflammation reactions but also help to minimize and recover the symptoms of inflammation. The use of such medicinal plants for herbal drug synthesis will improve the course of medication as being herbal the medicines impart least or no side effects on the consumer, while the studies have revealed the side effects caused due to medication with the non-steroidal anti-inflammatory drugs. Use of such herbal medicines could help overcome such harmful side effects as well as being natural its consumption feels safe to the consumer as well. The knowledge about importance of these plants as source of herbal medicine will also help in conserving such plants.

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