Original Article

GC-MS Analysis and Antibacterial Activity of Aerial Parts of *Quisqualis indica* Plant Extracts

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ABSTRACT

Background: Bacteria is an important group of human pathogens that causes various types of diseases. Plants are the traditional sources for many chemicals used as pharmaceutical biochemicals, fragrances, food colors and flavors in different countries especially in India. *Quisqualis indica* Linn. belongs to a family *Combretaceae* which is commonly known as Rangoon creeper has great medicinal values. **Objective:** To analyze the chemical constituents present in different solvent extracts of *Q. indica* using gas chromatography-mass spectroscopy (GC-MS) and evaluation of their antibacterial activities against human pathogens. **Method:** The dried aerial part of plant was extracted using three solvents like methanol, ethyl acetate and hexane at room temperature for 6 hours separately and antibacterial activity was carried out by broth dilution method. **Results:** 15, 12 and 18 compounds were confirmed by GC-MS qualitatively in methanol, ethylacetate and hexane extracts respectively. All extracts showed significant activity against four bacteria: *Escherichia coli, Klebsiella pnemoniae, Staphylococcus aureus* and *Staphylococcus pneumonia* which were comparable with ampicillin drug taken as positive control. **Conclusion:** The antibacterial result indicates that the aerial parts of plant have antibacterial activities due to presence of various phytochemical constituents. All the major components confirmed by GC-MS from different extract are biologically active molecule which indicates that the plant might have some medicinal properties.

Key words: Antibacterial activity, GC-MS analysis, Phytochemicals constituents, *Quisqualis indica.*

INTRODUCTION

Nature has been as source of medicinal agent for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Various medicinal plants have been used as a source of medicine for years in daily life to treat diseases all over the world.[1] *Q. indica* Linn. commonly known as Rangoon Creeper is an excellent vine for outdoor gardens belonging to family *Combretaceae*. Some medicinal properties of *Q. indica* Linn. has been documented in Ayurveda, Siddha, Unnani and other medicinal system.[2] Almost all of its parts are used individually or mixed with other ingredients as remedy to different ailments like antiflatulence, coughs, diarrhea,[3] body pains, antlelmintic, toothache,[4] and cardiovascular system,[5] Herbs that are rich in flavonoids, vitamin C or the carotenoids may enhance immune function.[6] A number of pharmacological studies have been reported on *Q. indica* Linn., immunomodulatory,[7-9] antibacterial, antioxidant,[10] antipyretic, anthelmintic,[11] antirrhumatic properties,[12] antiviral, antifungal,[13] anti-inflammatory, anti-staphylococcal and antiseptic properties[14] due to the presence of various phytochemical constituents all over the parts of plants. Its seeds and leaves are used for therapeutic purposes, like antigelmintoznoe tool, especially against tapeworm as well as a sedative. It has also been reported to be used successfully against stomach pain, cold, skin parasites, and rickettsia.[15] The leaf extract of *Q. indica* Linn. contain phytochemical such as quinone, flavonoids, Tannin, Phenolic,

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Saponin compound and cumarin, quinone and flvonoid.[16] The Flavonoids are a group of phenolics that are found in varying amounts in foods and medicinal plants which have been shown to exert anti-allergic, anti-inflammatory,[17] anti-microbial and antihepatotoxic activities.[18] One of the reason of using this plant in making many herbal products is its availability almost in every season and also it grows faster.[14]

The aim of present work was to analyze phytochemical constituents present in aerial parts of the plant extracts using methanol, ethylacetate and hexane by GC-MS and evaluate the antibacterial activity of these extracts.

MATERIALS AND METHODS

Materials

The plant *Q. indica* Linn. was collected from Gurgaon, Haryana (India) in November 2014. Methanol, ethylacetate and hexane (HPLC grade) were procured from Merck, India. The plant was identified by the scientist of the Institute.

Methods

The aerial parts of the plant was collected, washed, air dried in shadow and grinded by mixer grinder. After grinding, 5 gm of plant material was extracted in 50 ml of different solvents (methanol, ethylacetate, and hexane) separately at room temperature for 6 hours. The organic solvent was filtered by whatman filter paper till clear solution was obtained. Solvent was evaporated in a rotatory evaporator (Buchi, Switzerland) under reduced pressure (vacuum) at 40-50°C till complete dryness. For phytochemical analysis, 1000ppm solution of each extract was prepared separately by dissolving 1 mg dried plant material in 1ml of the same fresh HPLC grade solvent.

GC-MS analysis

The chromatographic procedure was performed using GC-MS model MSQP2010 (Shimadzu, Kyoto, Japan) with auto sampler. 1000 ppm solution in methanol, ethylacetate and hexane were prepared from three extracts (methanol, ethylacetate and hexane) and 1 µL of each extract was injected for analysis using DB -5MS column (30 meter × 0.25 mm, film thickness 0.25µm). Helium gas was used at flow rate1ml/min. as a carrier gas. The analysis was carried out using oven programming of initial temperature 50°C for 2 minutes followed by ramp rate of 20°C/minute up to 130°C followed by ramp of 12°C/min. to a temperature of 180°C, finally raised temperature to 280°C at 3°C per minute and hold for 15 minutes. The ion source temperature was set at 250°C. The injection port temperature was set as a 250°C and the total run time was 58.5 minute. The instrument was operated in electron impact (EI) mode with electron energy 70ev. Confirmation of analytes was done by SIM (selective ion mode) mode.[19,20]

Evaluations of antibacterial studies

Antibacterial studies of different plant extracts (methanol, ethylacetate, and hexane) of *Q. indica* Linn. were done against four bacteria species *E. coli, K. pneumonia*, *S. aureus* and *S. pnemoniae*. The study was carried out using the broth dilution technique by 2-fold serial dilution method to determine minimum inhibitory concentration (MIC). The procedure involves preparing two-fold dilutions of the antimicrobial agent (e.g. 6.25, 12.5, 25, 50, 100, 200 and 400µg/mL) in a liquid growth medium. The MIC is the lowest concentration of antimicrobial agent that inhibits growth of the organism as detected by the unaided eye. After getting the MIC value, the experiment has been done in triplicate nearest the MIC value. The antibacterial study was done from Micropharm Diagnostic Center, Gandhinagar, Gujarat, India.

Statistical Analysis

All the experiments were conducted in triplicate and statistical analyses were conducted using one way analysis of variance (ANOVA). The results were expressed as means of triplicate determinations ± standard deviation (SD). P values < 0.05 were considered as significant.

RESULTS

The plant extracts of *Q. indica* (methanol, ethylacetate and hexane extracts) were analyzed by GC-MS. The presence of components was confirmed by comparing mass spectra of analyzed components with standard mass spectra of NIST and Willey library. In the GC-MS analysis of *Q. indica,* 12 compounds were confirmed in ethyl acetate extract, 18 in hexane extract while 15 compounds in methanol extract. The active principles with their retention time (RT), molecular formula, molecular weight (MW), concentration (%), base ion and other fragment ion in methanol, ethylacetate and hexane extracts of *Q. indica* are presented in Table 1, 2, and 3 respectively. Chromatogram of methanol, ethylacetate and hexane extracts are shown in Fig. 1, 2 and 3 respectively and the structures of all the confirmed components are given in Scheme 1.

The major 15 compounds which were found in methanol extract are diethylphthalate (0.16%), isobutyl-o-phthalate (0.04%), methyl isohexadecanoate (0.18%), phytol isomer (8.69%), methyl tetradecanoate-12-Me (0.07 %), methyl linolelaidate (0.039%), 7,10,13-hexadecatrienal

Table 1: Phytochemicals detected in methanol extract.

RT	Compound name	Molecular weight	Molecular formula	% area	Base ion	Other fragments ion (m/z)
11.002	Diethylphthalate	222	$C_{12}H_{14}O_4$	0.16	149	75,105,177,222
14.444	Isobutyl-o-phthalate	278	$C_{16}H_{22}O_4$	0.04	149	57,104, 223
15.343	Methyl isohexadecanoate	270	$C_{17}H_{34}O_2$	0.18	74	55,97,143,171, 185,227,270
18.533	Methyl linolelaidate	266	$C_{17}H_{30}O_2$	0.39	67	81,96,123,150, 235
18.953	Phytol	296	$C_{20}H_{40}O$	8.69	71	57,81,95,111, 123,137
19.206	Methyl tetradecanoate,12-Me	256	$C_{16}H_{32}O_2$	0.07	74	55,97,143,157, 199,213
32.055	7,10,13-hexadecatrienal	234	$C_{16}H_{26}O$	1.17	79	67,108
35.084	trans Squalene	410	$C_{30}H_{50}$	7.70	69	55,95,149,191, 203
36.645	1- Dotriacontanol	466	$C_{32}H_{66}O$	0.59	57	83,97,125,153
37.353	Nerolidol isomer	222	$C_{15}H_{26}O$	0.15	69	109,81,135
39.794	Tocoferol	416	$C_{28}H_{48}O_2$	1.22	151	71,107,122,151, 191,416
41.474	Vitamin E acetate	472	$C_{31}H_{52}O_3$	3.19	165	57,91,121,136, 177,430
44.746	Stigmasterol	412	$C_{29}H_{48}O$	7.73	55	69,83,105,145, 173, 213,271,314
45.404	Viridiforol	222	$C_{15}H_{26}O$	3.44	69	81,93,109,161, 189
47.005	Cycloartenyl acetate	468	$C_{32}H_{52}O_2$	6.78	69	81,109,135, 149,175,393
	Unknown			58.5		

Table 2: Phytochemicals detected in ethylacetate extract.

RT	Compound name	Molecular weight	Molecular formula	% area	Base ion	Other fragments ion (m/z)
7.319	Dodecane	170	$C_{12}H_{26}$	0.08	57	71, 98
9.116	Heptadecane	240	$C_{17}H_{36}$	0.09	57	71, 99, 127, 155
10.138	Farnesene	204	$C_{15}H_{24}$	0.07	93	69, 107, 119
18.965	Phytol	296	$C_{20}H_{40}O$	8.00	71	57, 81, 95, 111, 123, 137
32.26	Octacosane	394	$C_{28}H_{58}$	1.05	57	71, 99, 127, 155, 183
35.087	trans squalene	410	$C_{30}H_{50}$	8.60	69	55,95,149,191, 203
36.709	Pentatriacotane	492	$C_{35}H_{72}$	4.10	57	71, 85, 113, 127, 155, 169
39.739	Gama – tocopherol	416	$C_{28}H_{48}O_2$	1.22	151	71, 107, 191
40.968	Tetratetraacotane	618	$C_{44}H_{90}$	12.3	57	71, 85, 113, 127, 155, 169, 197, 211, 225
41.474	Vitamin E acetate	472	$C_{31}H_{52}O_3$	3.93	165	57,91,121,136, 177,430
44.743	Stigmasterol	412	$C_{29}H_{48}O$	6.94	55	69, 81, 95, 105, 119,173, 215, 412
45.013	Heptacosane-1-chloro	414	$C_{27}H_{55}Cl$	7.04	57	71, 113, 141, 183
-	Unknown compound			46.58		

(1.17%), trans Squalene (7.70%), 1- dotriacontanol (0.59%), nerolidol isomer (0.15%) tocoferol (1.22%), vitamin E acetate (3.19%), sigmasterol (7.73%), viridiforol (3.44%) and cycloartenyl acetate (6.78%).

12 Compounds which were detected in ethylacetate extract by GC-MS are dodecane (0.08%), heptadecane (0.09%), farnesene (0.07%), phytol (8.00%), octacosane (1.05%),trans squalene (8.60%),pentatriacotane (4.10%), gama-tocopherol (1.22%), tetratetraacotane (12.3%), vitamin E acetate (3.92%), heptacosane-1- chloro (7.95%) and heptacosane-1-chloro (7.04%).

In hexane extract, 18 compounds were identified by GC-MS which are pentadecane (0.18%), farnesene (0.07%), farnesane (0.06%) 6-methyl octdecane (0.02%), heptadecane (0.16%), methyl palmitate (10.43%), methyl hexadeconate (1.93%), methyl linolate (8.69%), phytol (1.24%), methyl stereate (1.03%) octacosane (0.62%), trans-squalene (5.12%), pentatriacotane (3.17%), gama-tocopherol (0.77%), tetratetracontane (7.18%), vitamin

Table 3: Phytochemicals detected in hexane extract.

RT	Compound name	Molecular wt	Molecular formula	% area	Base ion	Other fragments ion (m/z)
9.120	Pentadecane	212	$C_{15}H_{32}$	0.18	57	71,99,127,155
10.140	Farnesene	204	$C_{15}H_{24}$	0.07	93	69,107,123
10.425	Farnesane	212	$C_{15}H_{32}$	0.06	57	71,99,127
10.69	6-methyl octdecane	268	$C_{19}H_{40}$	0.02	57	71,99,127
13.386	Heptadecane	240	$C_{17}H_{36}$	0.16	57	71,99,127
15.346	Methyl hexadeconate	270	$C_{17}H_{34}O_2$	1.93	74	55,57,87,97,115,129,143
18.575	Methyl palmitate	294	$C_{19}H_{34}O_2$	10.43	67	55,81,95,96,109,123,135,294
18.711	Methyl linolate	292	$C_{19}H_{32}O_2$	8.69	79	55,67,93,108,121,122,135,149,292
18.952	phytol	296	$C_{20}H_{40}O_2$	1.24	71	57,95,123,196
19.213	Methyl stereate	298	$C_{19}H_{38}O_2$	1.03	74	55,97,143,199,255,298
32.26	Octacosane	394	$C_{28}H_{58}$	0.62	57	71,99,127,155,183,211
35.088	Trans – squalene	410	$C_{30}H_{50}$	5.12	69	55,95,161,191,203,410
36.79	Pentatriacotane	492	$C_{35}H_{72}$	3.17	57	71,85,113,127,155,169
39.79	Gama – tocopherol	416	$C_{28}H_{48}O_2$	0.77	151	57,191,416,
40.96	Tetratetracontane	618	$C_{44}H_{90}$	7.18	57	71,85,113,127,155,169
41.488	Vitamin E acetate	472	$C_{31}H_{52}O_3$	3.92	165	57,91,121,136,177,430
44.752	Stigmesterol	426	$C_{30}H_{50}O$	4.67	55	105,145,173,213
45.018	Heptacosane-1-chloro	414	$C_{27}H_{55}Cl$	7.95	57	71,113,141,183
-	Unknown compound			42.79		

Table 4: Antibacterial activities of aerial parts of plant extracts using different solvents.

		Minimal inhibition concentration (µg/ml)			
S.No.	**Extract**	*E. coli.*	*Kl. pnemoniae*	*S. aureus*	*S. pnemoniae*
1	Ethyl acetate	100±1	50±0.5	25±0.2	50±0.3
2	Hexane	50±0.25	12.5±0.5	25±0.5	100±0.5
3	Methanol	12.5±0.5	50±1	25±1	100±0.75
4	Ampicillin drug (+ve control)	100±0.5	100±0.45	250±0.5	100±0.4

Values are mean of triplicate readings ± Standard Deviation (mean ± S.D).

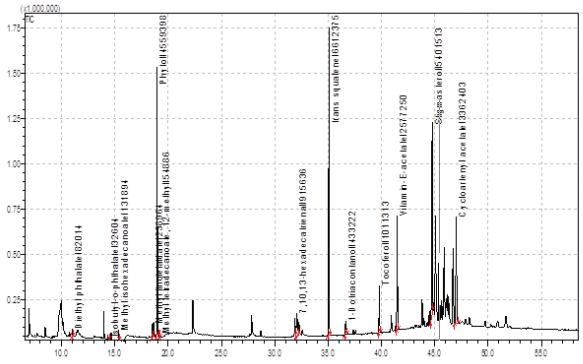


Figure 1: Chromatogram of methanol extract of *Q. indica*.

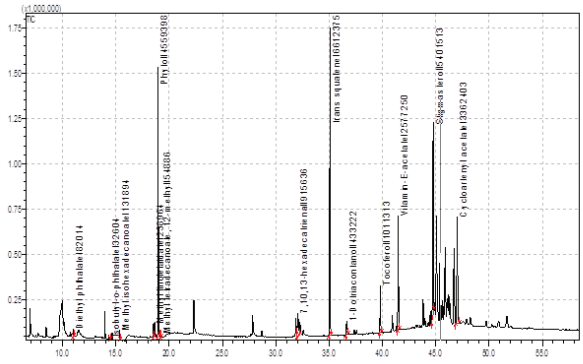


Figure 2: Chromatogram of ethylacetate extract of *Q. indica*.

E acetate (3.92%), heptacosane-1- chloro (7.95%) and stigmesterol (4.67%).

The maximum unknown chemical components were found in methanol extract i.e. 58.5% while minimum unknown chemical components were found in hexane extracts i.e. 42.79 %. In ethylacetate extract, the unknown chemical constituents were found to be 46.58 %.

The antibacterial activity observed among the various solvent extracts of *Q. indica* are summarised in Table 4. MIC values of ethylacetate extract against *E. coli*, *Kl. Pnemoniae, S. Aureus* and *S. pnemoniae* were found effective to control bacteria at 100µg/ml, 50µg/ml, 25 µg/ml and 50 µg/ml concentrations which are

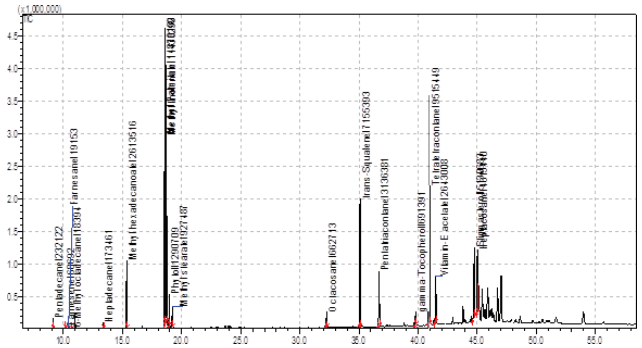
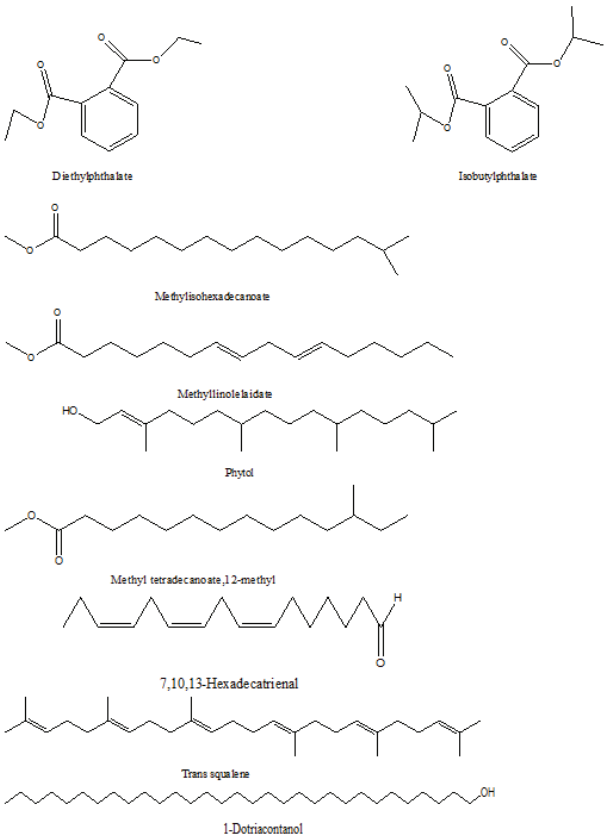
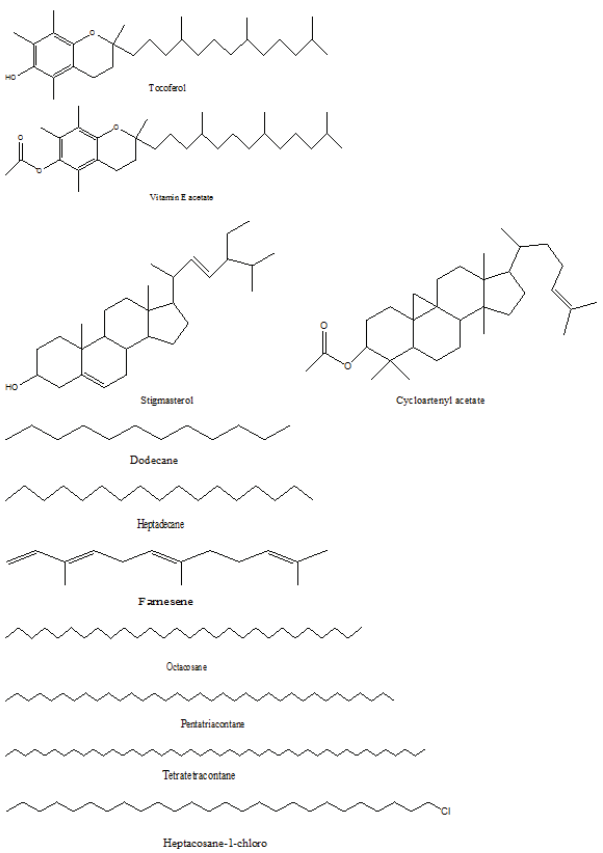
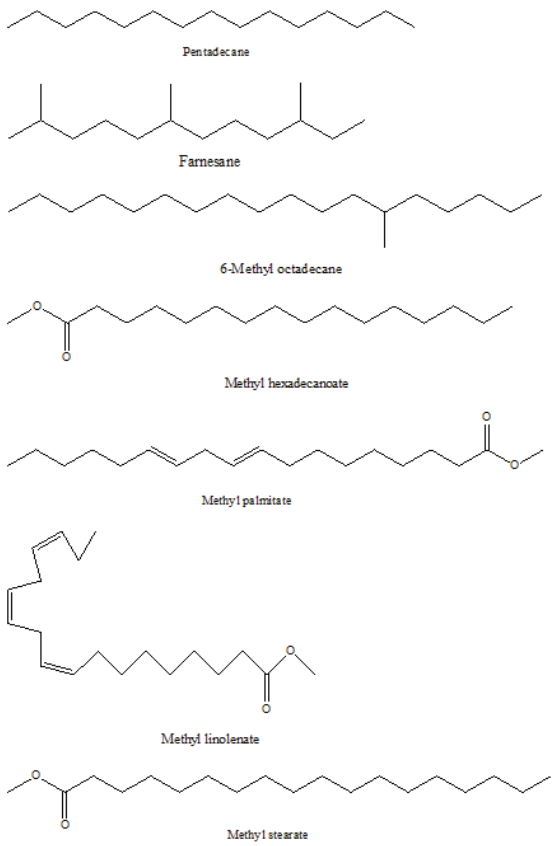


Figure 3: Chromatogram of hexane extract of *Q. indica*.







Scheme 4: Structures of confirmed components

comparable with Ampicillin (+ve control) having values 100μg/ml, 100μg/ml, 250 μg/ml and 100 μg/ml respectively.

MIC values of hexane against *E.coli*, *Kl. Pnemoniae, S. Aureus* and *S. pnemoniae* were found effective to control bacteria at 50μg/ml, 12.5μg/ml, 25 μg/ml and 100 μg/ml concentrations which are comparable with Ampicillin (+ve control) having values 100μg/ml, 100μg/ml, 250 μg/ml and 100 μg/ml respectively.

MIC values of methanol extract against *E.coli*, *Kl. Pnemoniae, S. Aureus* and *S. pnemoniae* were found effective to control bacteria at 12.5μg/ml, 50μg/ml, 25 μg/ml and 100 μg/ml concentrations which are comparable with Ampicillin (+ve control) having values 100μg/ml, 100μg/ml, 250 μg/ml and 100 μg/ml respectively.

DISCUSSION

In recent years, the interest for the study of the organic compounds found in plants and their activity has increased. The GC–MS is an ideal technique for qualitative and quantitative analysis of active components in plant. The aim of the present study was to confirm the phytochemicals present in the plant extracts and to evaluate their antibacterial activity. Diversity of medicinal plants and herbs containing various phytochemicals with biological activity can be of valuable therapeutic key. Different phytochemicals have been found to have a broad range of activities, which may help in protection against chronic diseases.[21]

The common constituents found in all the three extracts (methanol, ethylacetate and hexane) are phytol isomer (8.69% in methanol, 8.0% in ethylacetate and 1.24% in hexane), trans squalene (7.70% in methanol, 8.60% in ethylacetate and 5.12% in hexane), gama tocoferol (1.22% in methanol, 1.22% in ethylacetate and 0.77% in hexane), vitamin E acetate (3.19% in methanol, 3.93% in ethylacetate and 3.92% in hexane) and stigmasterol (7.73% in methanol, 6.94% in ethylacetate and 4.67% in hexane).

The GC-MS analysis of the methanol, ethylacetate and hexane extracts resulted many compounds which have many properties. The constituent phytol confirmed in all the solvents having antimicrobial, anticancer, anti-inflammatory activity[22] and is also used in the fragrance industry, cosmetics.[23] Constituent squalene is also having anti-cancer, anti-oxidant, anti-tumor, chemo-preventive, pesticidal and sun-screen properties.[24] Compound stigmasterol possesses potent antioxidant, hypoglycemic and thyroid inhibiting properties.[25]

Some constituents who were not present in methanol extract but common in other two solvents i.e. ethylacetate and hexane are farnesene (0.07% in ethylacetate and 0.07% hexane), octacosane (1.05% in ethylacetate and 0.62% in hexane), pentatriacontane (4.10% in ethylacetate and 3.17% in hexane), tetratetracontane (12.3% in ethylacetate and 7.18% in hexane) and heptacosane-1-chloro (7.04% in ethylacetate and 7.95% in hexane).

Phytol isomer in methanol extract, tetratetracontane in ethylacetate extract and methyl palmitate in hexane extract with their respective areas 8.69%, 12.3% and 10.43% were found to be major constituents. Tetratetracontane confirmed in ethylacetate extract has antioxidant and cytoprotective activities.[26] Methyl palmitate in hexane extract has an insect repellent activity.[27] Some antimicrobial activities of this plant have been previously studied. Hexane, carbon tetrachloride, Chloroform and aqueous fraction of methanolic extract of stem bark and aerial part of this plant were found effective against *S. aureus* and *E. coli*.[28,16] Methanol extracts of the flower of this plant was found effective against *E. coli*.[29,30]

It has been investigated that ethylacetate extract was found most effective against *S. aureus,* least effective against *E.coli* while *Kl. Pnemoniae* and *S. pnemoniae* showed same susceptibility against ethyl acetate extract. Hexane extract was found most effective against *Kl. Pnemoniae* while least effective against *S. pnemoniae* followed by *S. aureus* and *E.coli*. Methanol extract was found most effective against *E.coli* pathogen while least effective against *S. pnemoniae* follwed by *Kl. Pnemoniae* and *S. aureus*. Hexane and methanol extracts showed better efficiency to control selected pathogens followed by ethyl acetate extract. Authors have observed that all extracts of aerial part of *Q. indica* plant shows better antibacterial activities against selected bacteria as compared to ampicillin drug.

CONCLUSION

In the present study, total twenty seven chemical constituents have been confirmed from the three different extracts of the aerial parts of plant *Q. indica* by GC-MS analysis. The antibacterial study result indicated that the lowest MIC against *E.coli* was observed in methanol extract, against *Kl. Pnemoniae* was found in hexane extract and against *S. pnemoniae* was in ethylacetate whereas against *S. Aureus* the MIC value were same in all the three extracts. The presence of various phytochemical constituents justifies the use of plant for various ailments by traditional practitioners. Thus, this type of GC-MS analysis is the first step towards understanding the nature of active principles in this plant and this type of study will be helpful for further study. The result of this work indicated that the methanol

and hexane are better solvents than ethylacetate for the extraction of active ingredients of this plant. . Further studies are needed on these extracts in order to isolate, identify, characterize and elucidate the structure of these compounds.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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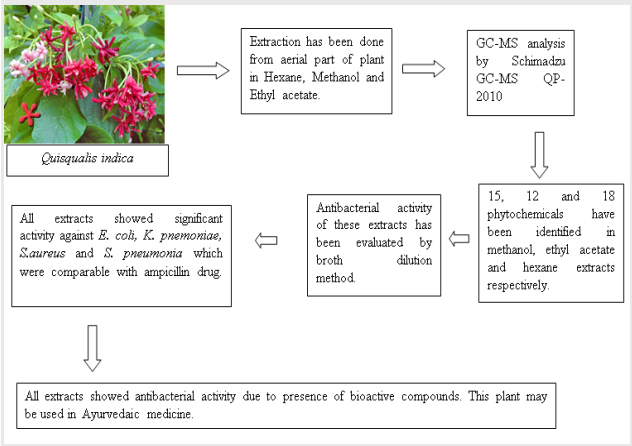
PICTORIAL ABSTRACT



SUMMARY

- *Quisqualis indica* Linn. belongs to a family *Combretaceae* which is commonly known as Rangoon creeper. It has great medicinal values.
- A number of pharmacological studies have been reported on *Q. indica* Linn. like immunomodulatory, antibacterial, antioxidant, antipyretic, anthelmintic, antirrhumatic properties, antiviral, antifungal, anti-inflammatory, anti-staphylococcal and anti-septic properties.
- Our objectives are to find out the presence of phytochemical constituents in aerial parts of the plant extracts using methanol, ethylacetate and hexane by GC-MS and their evaluation of antibacterial activity of these extracts.
- 15, 12 and 18 compounds were confirmed by GC-MS qualitatively in methanol, ethylacetate and hexane extracts respectively.
- All extracts showed significant activity against four bacteria: *Escherichia coli, Klebsiella pnemoniae, Staphylococcus aureus* and *Staphylococcus pneumonia* which were comparable with ampicillin drug taken as positive control.

About Authors

Akriti Agarwal: Has done her PhD in chemistry from University of Lucknow, in 2008. Her doctoral research is focused on Synthesis of biologically active compounds and biosynthesis of phytochemicals of Indian medicinal plants. At present she is working as a Research Associate and she analyses pesticide residues in food commodities by using GC-MS, GC-ECD and GC-FPD. She has worked as a Research Associate in the project "National Monitoring of Persistent Organic Pollutant Pesticides and PCBs in the Environment" under Global Monitoring Plan (GMP) at Indian Agricultural Research Institute (IARI) Pusa, New Delhi.

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