J. Indian bot. Soc. (2022) Vol. 102 (4) : 294-305
https://doi:10.5958/2455-7218.2022.00042.0

e-ISSN:2455-7218, ISSN:0019 - 4468
www.indianbotsoc.org

RESEARCH ARTICLE

Phytochemical profiles of leaf extracts of *Rotheca serrata* (L.) Steane & Mabb: a medicinal herb of Assam

Seema Khakhalary and Silistina Narzari*

Abstract The herb *Rotheca serrata* (L.) Steane & Mabb (*R. serrata*) locally known as Nangal Bhanga is a medicinal herb of Assam, India. It is broadly used in traditional medicine systems of Assam for curing various ailments including hepatitis, ulcer, diabetes and cancer. Through the present work it was intended to investigate the phytochemical constituents antioxidant potential and bioactive compounds of *R. serrata* leaves. Crude extracts were obtained through Soxhlet extraction, using solvents of increasing polarity, i.e., hexane, chloroform and methanol. Antioxidant activities including 2, 2-diphenyl-1-picryl hydrazyl (DPPH) free radical scavenging and H_2O_2 assays were performed using UV-Vis Spectrophotometer. Total phenolics, tannins and flavonoid content were estimated following standard protocols for quantitative phytochemical analysis. Gas Chromatography–Mass Spectrometry (GC–MS) was used to identify bioactive compounds that would account for the above recorded activities. Preliminary screening of phytochemicals indicated the existence of alkaloids, flavonoids, phenols, tannins. Higher concentrations of antioxidants, phenolics, tannins and flavonoids were extracted in methanol solvents compared to the other two solvents. The GC–MS analysis led to identification of 20 potential bioactive compounds of which 7 bioactive compounds were detected in methanol, 7 in hexane and 6 in chloroform extract. Bioactive compounds identified from leaves of *R. serrata* are reported for biological activities like antioxidant, anticancer, anti-tumor and chemo-preventive properties. Findings of this study indicate that methanol extract is a potent solvent for phytochemical extraction and analysis. Further, our study also suggests that isolation and elucidation of these bioactive compounds may play a vital role to find a new drug in near future.

Keywords: Antioxidant, Bioactive, Extract, Medicinal, Phytochemicals

Abbreviations

GC-MS: Gas Chromatography and Mass Spectroscopy
H_2O_2: Hydrogen Peroxide
R. serratum: Rotheca serratum
UV-Vis: Ultra Violet- Visible
GUBH: Gauhati University Botanical Herbarium
g: Gram
ml: Millilitre
TPC: Total Phenolic Content
TFC: Total Flavonoid Content
$AlCl_3$: Aluminium chloride
NaOH: Sodium Hydroxide
SD: Standard Deviation
SPSS: Statistical Package for the Social Sciences
OD: Optical Density
Ic50: Inhibitory Concentration

Introduction

Use of plants as a source of medicine is practised and is passed on through generations among many populations around the globe. So it forms an important component of the health care system. Assam is enriched with plant diversity and several plants have been used traditionally by Assamese people for therapeutic potentials. Plants used in traditional medicines usually contain a wide range of bioactive compounds that can be used to treat various infectious and chronic diseases (Duraipandiyan *et al.* 2006). Bioactive compounds can be detected through preliminary phytochemical screening tests. The result acquired from preliminary phytochemical screening may aid in discovering novel drugs that come from natural sources.

R. serrata called Nangol bhanga in Assamese is an important medicinal plant belonging to family Lamiaceae. *Rotheca serrata* (L.) scientifically classified as

✉ Silistina Narzari
nsilistina@gmail.com

Department of Biotechnology, Bodoland University, Kokrajhar- 783370, Assam, India

Received : 08 February 2022 Accepted : 18 March 2022 Published online : 24 November 2022

Clerodendrum serratum earlier was placed under family Verbanaceae. Phylogenetic analysis of its mitochondrial DNA shifted it to the Lamiaceae family (Steane *et al.* 1997). Traditionally, this plant finds its wide applicability in ethnomedicines of Assam. Keshava (1994) reported the use of *R. serrata* roots in medicinal preparations for treating numerous disorders like asthma, bodyache, bronchitis, cholera, dropsy, eye disorder, fever, inflammations, malaria, ophthalmic, rheumatism, snakebite, tuberculosis, ulcers and wounds. Owing to its biological activities like anti-inflammatory and antipyretic activities, the use of *R. serratum* has been reported for treating diseases as typhoid, cancer, jaundice and hypertension (Mukesh *et al.* 2012). Saha *et al.* (2012) and Kar *et al.* (2014) informed about the analgesic and anti-diabetic potentials of its leaves.

Other scientific report published on extracts and formulations revealed anti-asthmatic, mast cell stabilization and anti-allergic effects in roots of *R. serrata.* Studies on pharmacological activities also include hepatoprotective, anti-oxidant, anti-inflammatory and anticancer potential (Acharya *et al.* 2014). Various phytochemicals including Apigenin-7-glucoside, (7-(β-D-glucopyranosyloxy)-5-hydroxy2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one have been previously isolated from the root of *R. serrata* (L) Moon (Bhujpal *et al.* 2010) and D-mannitol, stigmasterol, oleanolic acid, ferulic acid, lupeol, and ursolic acid (Kumar and Niteshwar 2013).

The study was undertaken to screen phytochemicals, evaluate in-vitro antioxidant activities and identify and characterize bioactive compounds in *R. serrata* extracts. This study will provide scientific validation for the therapeutic practice of using *R. serrata* leaves in traditional medicine systems of Assam. Identification of bioactive compounds in *R. serrata* essentially may lead to further dissemination of knowledge on its biological and pharmacological studies.

Materials and methods

Chemicals

All chemicals and solvents used were of analytical grade and were purchased from Merck (Germany).

Collection of Plant material

Fresh leaves were collected in the month of January 2021 during the morning hours. The collected plant material was authenticated by the Gauhati University Botanical Herbarium (GUBH), Gauhati University, Assam. A voucher specimen of *Rotheca serrata* (L.) Steane & Mabb bearing accession number 18926 was then submitted to GUBH.

Preparation of Plant extract

Fresh leaves were collected, cleaned, washed and dried under shade for three weeks. The air dried leaves were crushed and pulverized using a clean and sterile electric grinder. 20g of powdered leaves were extracted successively in 200ml of hexane, chloroform and methanol solvent at room temperature for about 24 hours using Soxhlet apparatus. The solvents were evaporated in a rotary vacuum evaporator (Model no.#EV11) to obtain crude extracts.

Finally, the yield percent of crude extract was calculated by the standard formula of Alebiosu and Yusuf (2015).

$$\text{Yield Percent (\%)} = a/b \times 100$$

Where, a = dry weight of extract obtained
b = initial weight of powdered material

Phytochemical Screening

The plant extracts were subjected to phytochemical screening tests by following standard protocols of Evans (2009), Harborne (1998) to confirm the presence of phytochemicals.

Determination of Total Phenolic Content

To evaluate total phenolic content (TPC) in *R. serrata* crude extracts the Folin-Ciocalteu reagent method of Shukla *et al.* (2014) was followed. For the analysis, 0.2 mL of extract (1mg/ml) was added to 2.5mL of 10% Folin-Ciocalteu reagent and then neutralized using 2ml of 7.5% sodium carbonate. The reaction mixture was then incubated in dark at normal room temperature for 30mins. Absorbance value was measured at 765nm wavelength using a double beam UV-Vis spectrophotometer (UV Analyst-CT8200). The total phenolic content as mean SD (n=3) were calculated from the linear regression equation of gallic acid standard plot. The results are expressed as mg/g gallic acid equivalent (GAE) of dry extract.

Determination of Total Flavonoid Content

Total flavonoid content in *R. serrata* extracts was determined by the procedure described by Alhakmani *et al.* (2013). Calibration curve was constructed using quercetin as standard. 0.2mL of plant extract (1mg/ml) was diluted with 5ml of distilled water. To it 0.5ml of 5% sodium nitrite solution was added. After 5 mins, 0.6ml of 10% $AlCl_3$ solution was added. After another 6 mins, 2ml of 1M NaOH solution was added and final volume was adjusted to 3ml with distilled water. The solutions were thoroughly mixed and incubated for 15minutes. Absorbance value of the reaction mixture was measured at 510nm with double beam UV-Vis spectrophotometer (UV Analyst-CT8200) against blank. All the tests were performed in triplicates. Total flavonoid was calculated from the quercetin calibration curve. Results are expressed as mg quercetin equivalent per gram dry weight.

Determination of Total Tannin Content

The tannins were determined using the Folin-Ciocalteu method of CI and Indira (2016). To 0.1ml of plant extract (1mg/ml), 1ml of distilled water was added. To it 0.5 ml Folin–Ciocalteu reagent was added and mixed thoroughly. The mixture was alkalinized by adding 1ml of 15% (w/v) Na_2CO_3 and kept in dark for 30 minutes at room temperature. The absorbance of the tannic acid standard solutions as well as sample was measured after colour development at 700nm using the UV-VIS spectrophotometer (UV Analyst-CT8200). Results calculated using the calibration curve were expressed as mg/g equivalent of tannic acid.

Determination of Antioxidant Activity

The antioxidant activities of the plant extract vary with the solvent used for extraction. It is thus important to use different solvent extract for evaluating the effectiveness of the antioxidant. The antioxidant activity of all three solvent extract i.e. hexane, chloroform and methanol extract was determined using 2, 2-Diphenyl-1-Picryl-Hydrazyl Assay (DPPH) and Hydrogen Peroxide Assay (H_2O_2).

2, 2-Diphenyl-1-Picryl-Hydrazyl Assay (DPPH Method)

Free radical scavenging activity of the crude extracts were evaluated by using DPPH radical scavenging activity method of Alhakmani et al. (2013). Ascorbic acid was taken as the standard. Crude extracts and standard ascorbic acid solution of 1mg/ml of varying concentrations ranging from 50 to 250µg/mL were taken in separate test tubes. 2ml of 0.1mM DPPH prepared in methanol was added to each test tube. The solution was mixed and kept in dark at 37°C for 30mins. The decrease in absorbance of each solution was measured at 517nm using UV-Vis spectrophotometer (UV Analyst-CT8200). The solution used for the blank is methanol. Radical scavenging activity expressed as percentage inhibition of the extract and ascorbic acid were calculated using the standard formula:

% Inhibition = OD control - OD test / OD control × 100

The concentration of sample required to scavenge 50% of DPPH free radical (IC50) was calculated from the curve of percent inhibitions plotted against their respective concentrations.

Hydrogen Peroxide Scavenging Activity (H_2O_2)

The ability of leaf extracts to scavenge H_2O_2 was studied by the method of Nabavi *et al.* (2008). Different concentrations ranging from 50-250 µg/mL of crude extracts and standard ascorbic acid solution were taken in test tubes. To each test tubes, 0.6mL of H_2O_2 (40mmol/L) and 2ml of phosphate buffer (50mmol/L) (pH 7.4) was added. After 10 minutes, absorbance was measured at 230nm against a blank solution containing phosphate buffer. The percentage of H_2O_2 scavenged was calculated using following formula:

H_2O_2 scavenge (%) = OD control - OD test/ OD control × 100

Gas Chromatography-Mass Spectroscopy (GC-MS)

GC separation of compounds was performed in Clarus 680 GC from Perkin Elmer, USA and MS study in Clarus 600C MS from Perkin Elmer, USA. For compound separation in GC, 2µl of extract was taken and injected into GC system through autosampler with a split ratio of 10:1 in splitmode. The GC system was fitted with 60m length capillary column of 0.25mm diameter and film thickness of 0.25µm. The column composition was 5% of diphenyl, 95% of dimethylpolysiloxane with a mass range around 50-600amu. Mass Spectra of the compounds were constructed at 70eV in Electron Impact positive (EI+) mode. The programming of column oven temperature was fixed between 60°C to 300°C and was held for 10mins. The temperature for the injector was kept at 280°C. The carrier gas used was Helium of 99.99% purity and the flow rate was fixed at 1ml/min^{-1}. The total run time for the whole GC-MS run was 51.83 minutes (Hema *et al.* 2010).

Identification of Compounds

Interpretation on Mass-Spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST 2014). The spectrum of components obtained from our study was compared with the spectrum of known components already stored in the NIST library. Through this comparison name, molecular weight and structure of the unknown components in *R. serrata* extracts were thus ascertained (Hema *et al.* 2010).

Statistical Analyses

All statistical analyses were performed in SPSS 26.0 version software. Experimental measurements were carried out in triplicates and are expressed as average of three analysis ± standard deviation (SD).

Results

Yield % of the crude extract

The yield % for hexane, chloroform and methanol extract of *R. serrata* were 2%, 2.8% and 3.5% respectively in 25g of powdered material used.

Preliminary Phytochemicals Screening

Results of the preliminary phytochemical screening disclosed the presence of various phytochemicals. It showed the presence of major classes of secondary metabolites such as tannins, flavonoids, phenolics, steroids, phytosterols etc. in all the extracts (Table-1). However, saponins, oils and fats were absent in chloroform and hexane extracts but present in

Table-1: Phytochemical components of solvent extracts of *R. serrata* based on preliminary screening.

Phytochemical constituents	Test	Methanol Extract	Chloroform Extract	Hexane extract
Alkaloids	Mayer's test	+	-	+
Tannins	Ferric chloride test	+	+	+
Saponins	Foam test	+	-	-
Phenolics	Ferric chloride test	+	+	+
Flavonoids	Alkalinereagent test	+	+	+
Phytosterols	Liebermaan Burchard's test	+	+	+
Steroids	Salkowski's test	+	+	+
Terpenoids	Salkowski's test	-	+	+
Oils and fats	Spot test	+	-	-

(+) =Detected; (-) =Not detected.

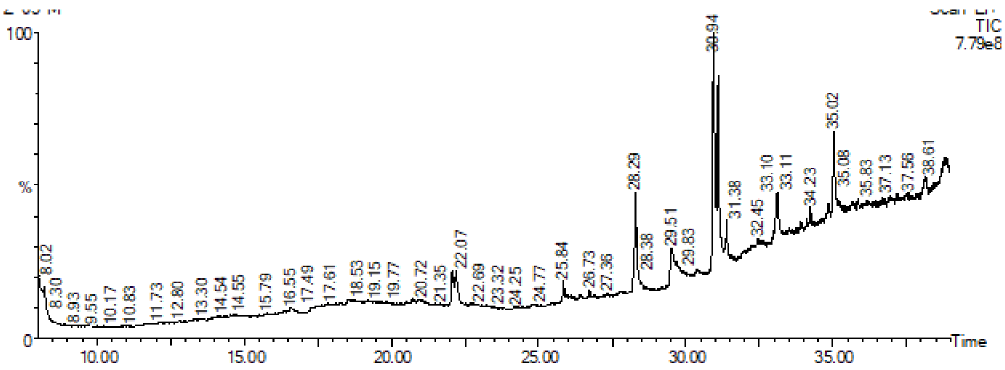


Figure 1: L GCMS chromatogram of methanolic extract of *R. serrata* leaves.

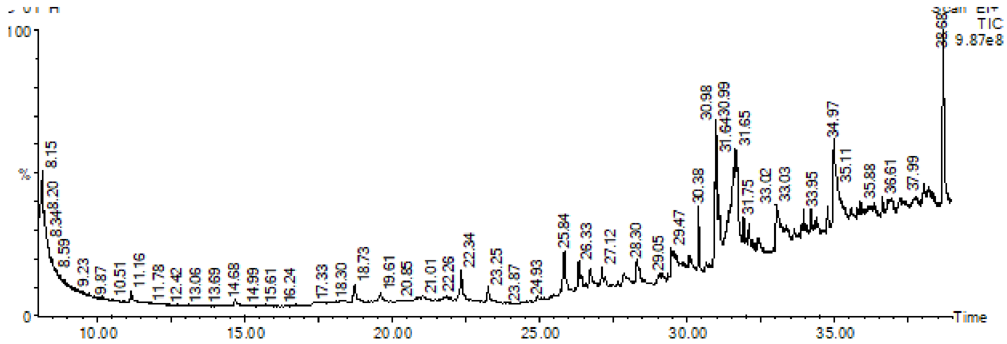


Figure 2: L GCMS chromatogram of hexane extract of *R. serrata* leaves.

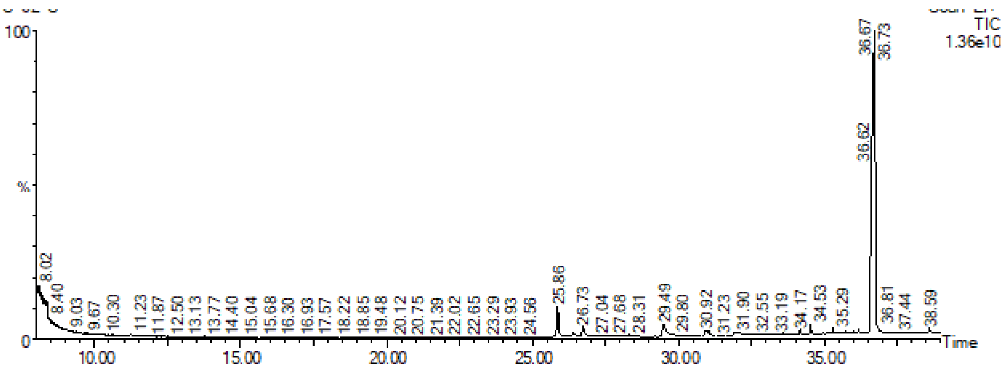


Figure 3: L GCMS chromatogram of choloroform extract of *R. serrata* leaves.

methanol extract.

Quantitative Phytochemical Screening

Total Phenolic Content (TPC)

The Total Phenolic Content (TPC) of leaf extracts is expressed in terms of GAE. The linear regression equation obtained from the standard plot of gallic acid was y = 0.004x + 0.059, R^2 = 0.984 where y is absorbance and x is the amount of gallic acid in µg. The TPCs were calculated from the standard plot (Table-2).

Total Flavonoid Content (TFC)

The Total Flavonoid Content (TFC) of *R.*

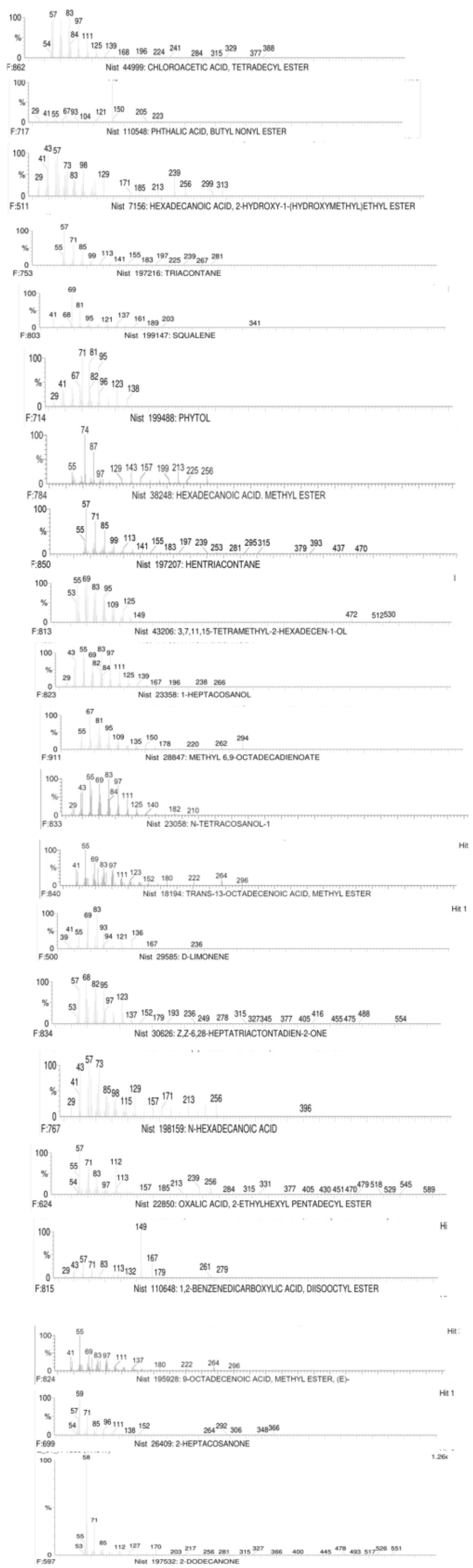


Figure 4: Mass spectrum of bioactive compounds present in *R. serrata* leaves extract

serrata leaf extracts is expressed in terms of Quercetin Equivalent (QE). The linear regression equation y = 0.004x + 0.714, R^2 = 0.917 where y is absorbance and x is the amount of quercetin in µg was obtained from standard plot of quercetin. The TFC was calculated from the standard plot and is presented in Table-2.

Total Tannin Content (TTC)

The Total Tannin Content (TTC) of leaf extracts is expressed in terms of Tannic Acid Equivalent (TAE). The linear regression equation y = 0.074x - 0.517, R^2 = 0.901 where y is absorbance and x is the amount of tannic acid in µg were obtained from the standard plot of tannic acid. The TTCs were calculated from the standard plot and is shown in Table-2

Among the three extracts, maximum amount of phytochemicals tested i.e phenolic, flavonoid and tannin were found in methanol extract followed by chloroform and hexane extracts.

Antioxidant Activity

The antioxidant activity of three crude extracts of *R. serrata* leaves was studied by commonly used radical scavenging methods such as DPPH and H_2O_2. The scavenging effect of leaf extracts on the DPPH and H_2O_2 free radicals were calculated from their absorbance. Inhibitory concentrations (i.e. IC50 value) of each extracts were calculated from the calibration curve of their percentage inhibition and results were compared with the standard ascorbic acid. The highest antioxidant activity expresses the lowest IC50 (Table-3).

Methanol extract showed lowest IC50 value in DPPH radical scavenging activity compared to hexane and chloroform extracts. But, the H_2O_2 scavenging activity of extracts were found in the following order of chloroform>methanol> hexane>. Both the assays have lower antioxidant capacity compared to ascorbic acid (Standard).

GCMS Analysis of the Plant Extract

Gas chromatography mass spectroscopy analysis was carried out to identify bioactive

Table-2. Total phenolic, flavonoid and tannin content of the crude extracts of *R. serrata*.

Crude Extract	Total phenolic content (mg of GAE/g dry extract)	Total flavonoid content (mg of QE/g dry extract)	Total tannin content (mg of TAE/g dry extract)
Hexane	30.35±2.24	16.10±0.145	7.50±0.015
Chloroform	56.76±1.75	21.43±0.098	7.71±0.02
Methanol	73.41±1.66	27.41±0.635	12.68±0.032

Mean values ± standard deviations of triplicate determinations are reported.

Table-3: IC50 value (in µg/ml) of R. serrata extracts from DPPH and H_2O_2 scavenging assay

Assays	Hexane	Chloroform	Methanol	Ascorbic Acid (Standard)
DPPH	226.95±0.997	220.96±2.096	156.37±0.910	115.30±1.35
H_2O_2	288.64±4.976	202.36±2.835	224.58±1.123	161.13±1.84

compounds in *R. serrata* leaf extracts. A total of 20 bioactive compounds were identified from the GC-MS analysis. Out of which, 7 compounds were detected in hexane extract, 6 in chloroform and 7 in methanol extract. The GC-MS chromatogram and Mass Spectrum of bioactive compounds obtained from methanol, hexane and chloroform extract are presented in (Figures. 1, 2, 3 and 4) respectively while the chemical constituents along with their retention time (RT), molecular formula, molecular weight (MW), and peak are a percentage and Pub Chem ID are presented in Table-4. The mass spectrum profile of GC-MS confirmed the presence of bioactive compounds with retention time ranging between 22.34 minutes - 35.02 minutes.

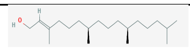
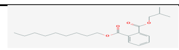
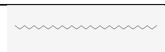
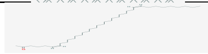
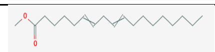
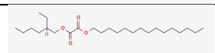
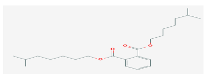
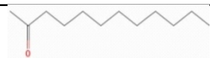
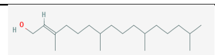
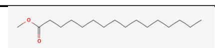
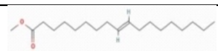
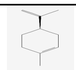
Discussion and conclusion

Crude extracts obtained from *R. serrata* leaves were observed for colour formation. The colour of hexane extract was yellow where as both chloroform and methanol extract appeared dark green. The dry weight and final weight of *R. serrata* extracts were significantly affected by solvent polarity used for extraction. The yield percentage of the extracts so calculated divulged that methanol extract had high extraction value as compared to chloroform and hexane. When the extracts were screened for phytochemicals, methanol extract contained higher amount of phytochemicals. So, methanol was found to be more potent for extracting phytochemicals compared to hexane and chloroform. It indicates that the leaf extract contained more polar than non-polar compounds. The study revealed that differences arise in the composition of phytochemicals due to variations in solvent polarities used for sample extraction.

Based on the results of phytochemical screening, the total phenolic, flavonoid and the total tannin content were estimated. The quantitative tests results revealed that there are wide variations in the phytochemical contents of the extracts (Table-2). Quantitative data obtained from standard calibration curve and calculated by linear regression equation expressed that methanolic extract contained significant quantity of phenol, flavonoid and tannin (i.e. 73.4mg of GAE/g extract, 27.41mg of QE/g extract, and 12.68mg of TAE/g extract) in comparison to hexane and chloroform extracts. Our study, ascertained that methanol is a superior solvent for isolating polyphenolic compounds compared to hexane and chloroform. Plant phenolics constitute one of the major groups of compound acting as primary antioxidants and free radical terminators.

The total antioxidant activity of the plant extracts were also evaluated using DPPH and H_2O_2 scavenging assay. Ascorbic acid was used as positive control for both the assays. IC50 value was calculated to evaluate the total antioxidant activity from the linear regression equation. A lower IC50 value corresponds to higher effectiveness of the antioxidant. In the present study, methanol extract showed maximum ability in DPPH radical scavenging activity compared to other solvent extract which was measured by the lowest IC50 value, but it has lower antioxidant capacity compared to ascorbic acid (Standard). The IC50 value in (µg/ml) of the extracts were found in the order of Methanol>Chloroform>Hexane (Table-3). But in H_2O_2 assay, the chloroform extract exhibited the highest antioxidant activity followed by methanol and hexane extract. These variances result from the point that each method is established on the production and use of various radicals and species that are actively involved in oxidative process by different mechanisms. The variation might be

Table 4: Bioactive compounds identified in hexane, chloroform and methanol extract of *R. serrata*.

	Retention time (min)	Compound Name	Molecular formula	Molecular Weight	Peak Area (%)	2D Structure of compounds	Pub Chem ID
Hexane extract	22.34	Triacontane	$C_{30}H_{62}$	422	1.179		12535
	25.84	Phytol	$C_{20}H_{40}O$	296	2.296		5280435
	30.98	Phthalicacid,butylnonylester	$C_{21}H_{32}O_4$	348	6.099		6423814
	31.64	Hentriacontane	$C_{31}H_{64}$	436	11.974		12410
	33.03	Hexadecanoicacid,2-hydroxy-1-(hydroxylmethyl)ethyl ester	$C_{19}H_{38}O_4$	330	2.902		341733478
	34.98	Chloroaceticacid,tetradecylester	$C_{16}H_{31}O_2Cl$	290	8.220		519540
	38.68	Squalene	$C_{30}H_{50}$	410	6.320		638072
Chloroform extract	26.72	Z,z-6,28-heptatriactontadien-2-one	$C_{37}H_{70}O$	530	1.443		5365964
	29.49	N-hexadecanoicacid	$C_{16}H_{32}O_2$	256	4.186		16213579
	30.92	1-heptacosanol	$C_{27}H_{56}O$	396	0.766		74822
	31.90	Methyl6,9-octadecadienoate	$C_{19}H_{34}O_2$	294	0.891		549027
	34.52	Oxalicacid,2-ethylhexylpentadecylester	$C_{25}H_{48}O_4$	412	0.637		6420799
	36.69	1,2-Benzenedicarboxylicacid,diisooctylester	$C_{24}H_{38}O_4$	390	69.89		33934
Methanolic extract	22.07	2-Dodecanone	$C_{12}H_{24}O$	184	1.914		22556
	25.84	3,7,11,15-tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	3.553		5366244
	28.29	Hexadecanoicacid,methylester	$C_{17}H_{34}O_2$	270	1.983		8181
	29.51	N-tetracosanol-1	$C_{24}H_{50}O$	354	7.883		10472
	30.94	9-octadecenoicacid,methylester	$C_{19}H_{36}O_2$	296	3.372		5280590
	31.38	Trans-13-octadecenoicacid,methylester	$C_{19}H_{36}O_2$	296	15.453		5364506
	33.10	D-limonene	$C_{10}H_{16}$	136	1.681		440917

the due to the complex nature of phytochemicals present in the extracts or the solvent used for extraction, etc (Rakholiya *et al.* 2011). It is thus important to perform several analytical methods for evaluating the effectiveness of antioxidants present in the plant. Phytoconstituents like flavonoids and phenolic compounds, commonly found in plants have been reported for its multiple biological effects, one of which is the antioxidant property (Tungmunnithum *et al.* 2018). Hence, in our study, the observed antioxidant activity might have ascended from the presence of phenolics and flavonoid contents in the extract of *R. serrata*. Tannins have also been reported to be associated in traditional treatment of ulcerated tissue and for its remarkable activity in cancer prevention (Batista *et al.* 2012). The optimum yield of tannin content of *R. serrata* leaves corroborates its traditional usage in treatment of cancer. Phytoconstituents having biological activities which include anti-inflammatory, antioxidant, antibacterial, antitumor, antidiabetic, hypercholesterolemia activities have been identified in the present study. The presence of these bioactive compounds stakes the reported utilization of *R. serrata* for various ailments. Based on abundance, the top three compounds present in the hexane extract were Hentriacontane (11.97%), Chloroacetic acid, tetradecyl ester (8.22%) and Squalene (6.32%). 1, 2-Benzene dicarboxylic acid, diisooctyl ester (69.89%) followed by N-hexadecanoic acid (4.18%) and Z, z-6,28-heptatriactontadien-2-one (1.443%) were the major compounds found in chloroform extract. Trans-13-octadecenoic acid methyl ester (15.453%), N-tetracosanol-1 (7.883%) and 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol (3.553%) were the top major bioactive compounds found in the methanol extract.

Most of the compounds identified in *R. serrata* leaves through GCMS analysis are biologically active compounds. Hentriacontane, the top compound obtained in the hexane extract is reported for its various pharmacological effects including antitumor activity (Kim *et al.* 2011). Chloroacetic acid, tetradecyl ester found in hexane extract (8.220%) is known for its antioxidant properties (Shyam and Suresh 2013). Triacontane is reported for its antibacterial, antidiabetic and antitumor activities (Mallick and Dighe 2014). The compound Squalene has antioxidant, chemopreventive, anti-tumour and hypercholesterolemia activities (Gunes 2013). N-hexadecanoic acid have several biological activities like antioxidant, 5 alpha reductase inhibitor, anti-fibrinolytic, antimicrobial activity, hypercholeseromic nematicide, pesticide, antiandrogenic flavor, and haemolytic property (Starlin *et al.* 2019). 3,7,11,15-tetramethyl-2-hexadecen-1-ol is a diterpene associated with biological activities like antimicrobial, anti-inflammatory, anticancer and diuretic activities (Hamidi *et al.* 2016). Al-Abd *et al.* (2015) reported 1-heptacosanol to have antimicrobial & antioxidant property. Z, z-6, 28-heptatriactontadien-2-one has vasodilatory property (Mallikadevi *et al.* 2012). 1, 2-Benzene dicarboxylic acid, diisooctyl ester the top major bioactive compound obtained in the study has antioxidant property (Li *et al.* 2012). Phytol was reported to exhibit antioxidant and antinociceptive effects (Santos *et al.* 2013). Phytol has been reported with cytotoxic activities against breast cancer cell lines (MCF7) (Pejin *et al.* 2014). Hexadecanoic acid, methyl ester possesses anti-bacterial and antifungal properties (Chandrasekaran *et al.* 2011). Phthalic acid, butyl nonyl ester is not known for any biological activities yet. Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester has antioxidant activity (Arora and Kumar 2018). The minor bioactive compound detected in chloroform extract namely Oxalic acid, 2-ethylhexyl pentadecyl ester (0.637%) has not been informed for any biological activities. Methyl 6, 9 octadecadienoate has anti-oxidant activity (Berdeaux *et al.* 1998). 2-Dodecanone detected in methanol extract has insecticidal and repellent activity (Wang *et al.* 2019). N-tetracosanol-1, an alcoholic compound present in methanolic extract is known for its antioxidant properties (Lakshmi and Nair 2017). Trans-13-octadecenoic acid, methyl ester has anti-inflammatory, antiandrogenic, anticancerous, dermatitigenic, hypocholesterolemic, anemiagenic and insectifuge properties (Krishnamurthy and Subramaniam 2014). 9-octadecenoic acid, methyl ester has anticancer activity (Asghar *et* *al.* 2011). Anandkumar *et al.* (2020) informed about the cardioprotective, hepatoprotective and anti-carcinogenic activities of D-limonene.

The above mentioned bioactive compounds from extracts of *R. serrata* leaves hold the reported biological activities. Triacontane in hexane extract justifies the reported anti-diabetic activity of the leaves. The reported anticancer activity of the Hexadecanoic acid, methyl ester, Phytol, 3,7,11,15-tetramethyl-2-hexadecen-1-ol and Squalene as informed by various authors supports the reported use of *R. serrata* in cancer treatment.

Based on the above results, it can be concluded that *R. serrata* is a good source of phytochemicals with potent pharmacological properties. It is also evident that methanol extract have superior antioxidant capacity compared to other solvent extract used in this study. 20 bioactive compounds identified in *R. serrata* leaves can contribute effective biological activities like antioxidant, antimicrobial, anticancer, anti-inflammatory, chemopreventive, anti-tumour, hypercholesterolemia activities, 5 alpha reductase inhibitor, anti-fibrinolytic activity if utilised properly. Biological activities of bioactive compounds in *R. serrata* leaf extract support the reported use of this plant in treating various ailments. Identification of bioactive compound in *R. serrata* can serve as the basis for determining possible health benefits of this plant. This study exposes new horizons for further biological and pharmacological research for better exploration of bioactive compounds from plants and their establishment for proper utilization in healthcare systems.

Acknowledgement

Acknowledge the financial support of the National Fellowship for Schedule Tribe (NFST) granted by Ministry of Tribal Affairs, Govt. of India to Seema Khakhalary. Authors are also grateful to Indian Institute of Technology (IIT) Biotech Park, Guwahati for assisting in spectral analysis.

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