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Original Article

# GC-MS Profiling, *in vitro* Antioxidant and Antimicrobial Activities of *Kaempferia parviflora* Wall. ex. Baker Rhizome Extract

**Rameshor Singh Atom[1,2,*], Shaukat Ali M Shaikh[3,4], Kishorjit Maharabam[2], Rakhi Khunjamayum[5], Dini Ahanthem[2], Warjeet Singh Laitonjam[2,*], Raghumani Singh Ningthoujam[6], Amit Kunwar[7]**

[1]Department of Chemistry, Waikhom Mani Girls' College, Thoubal, Manipur, INDIA.
[2]Department of Chemistry, Manipur University, Imphal, Manipur, INDIA.
[3]School of Chemical Sciences, Centre for Excellence in Basic Sciences, Mumbai University, Mumbai, Maharashtra, INDIA.
[4]Department of Atomic Energy, Mumbai University, Mumbai, Maharashtra, INDIA.
[5]Department of Biochemistry, Manipur University, Imphal, Manipur, INDIA.
[6]Chemistry Division, Bhabha Atomic Research Centre, Mumbai, Maharashtra, INDIA.
[7]Radiation and Photochemistry Division, Bhabha Atomic Research Centre, Mumbai, Maharashtra, INDIA.

## ABSTRACT

**Bakground:** *Kaempferia parviflora* (zingiberaceae family) rhizomes are used for various pharmacological applications. The present study is aimed for putative identification of phyto-constituents in the chloroform extract of the rhizome of *Kaempferia parviflora* and to correlate with bioactivities like antioxidant and anti-microbial/fungal activities. **Methods:** Rhizome extract was prepared using Soxhlet extractor and undergone the phytoconstituents analysis of it by gas chromatography–mass spectrometry (GC-MS). **Results:** Identified twenty-five compounds, out of which 4H-1-Benzopyran-4-0ne,5,7-dimethoxy-2-phenyl and Hydroxyurea showed the maximum and minimum relative abundance of 76.4% and 0.002% respectively. By agar well diffusion method, the extract (25 µg/ml) exhibited highest anti-bacterial potential against *Micrococcus luteus* (MTCC 106) (gram-positive bacteria) and anti-fungal potential against plant pathogen *Rhizoctonia solani* (MTCC 4633). The extract also showed the potent scavenging activities of 2,2-diphenyl-1-picryl-hydrazine (DPPH) and 2,2′-Azino-bis (3-Ethylbenzothiazoline-6-Sulfonic Acid) (ABTS) radicals with $IC_{50}$ values of 3.0 ± 0.1 mg/ml and 19.4 ± 1.2 µg/ml respectively. Subsequently, lipid peroxidation assay was also used for estimation of antioxidant activity of this extract. The extract (20 µg/ml) inhibited lipid peroxidation by 80.0 ± 4.5%. **Conclusion:** The outcomes of present study provide scope for isolation of bioactive compounds from *Kaempferia parviflora* for medicinal application.

**Keywords:** GC-MS, *Kaempferia parviflora*, Phytochemicals, Rhizome, Bioactivity.

**Correspondence**

**Dr. Rameshor Singh Atom**
Department of Chemistry, Waikhom Mani Girls' College, Thoubal-795138, Manipur, INDIA.

Email id: rameshoratom@gmail.com

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## INTRODUCTION

*Zingiberaceae*, or ginger family, consists of perennial herb, mostly terrestrial with aromatic, fleshy, tuberous rhizomes. The plant species offer several valuable products such as spices, food, dyes, perfume, medicines and aesthetics.[1] They are mainly available in tropical, subtropical areas of Asia and India. Till date about 22 genera and about 170 species of *Zingiberaceae* have been reported from India. Within India, North-Eastern region is a zone of greatest diversity of the family with about 19 genera and 88 species reported so far.[2] A few commercially important plant species of *Zingiberaceae* are *Elettaria cardamomum* Maton (cardamom), *Curcuma aromatica* Salisb. (kasturi turmeric), *Zingiber officinale* Rosc. (ginger), *Curcuma longa* L. (turmeric), *Curcuma amada* Roxb. (mango ginger), *Amomum subulatum* Roxb. (large cardamom), *Aframomum* spp., *Kaempferia* spp. etc. Generally, the rhizomes of this family are rich sources of essential oils, and are consumed as tonic and stimulant. Additionally, plants of *Zingiberaceae* are well known for their use in traditional medicine. Many terpenoid compounds obtained from the essential oils of *Zingiberaceae* plants have been reported to possess varied physiological activities like anti-microbial, anti-cancer, anti-arthritic, anti-oxidant, anti-inflammatory, anti-diabetic, neuroprotective, anti-HIV and larvicidal.[3-7]

*Kaempferia parviflora* is one such medicinal plant of *zingiberaceae* whose rhizomes have been used as a remedy for many ailments by the native people of Manipur, North-East, India since time immemorial without proper scientific formulations.[8] The plant is locally known as Singmu in Manipuri and Thai ginseng or Black Ginger in Thailand.[9] Medicines derived from the rhizomes of *Kaempferia parviflora* have been reported to use for inflammation, hypertension, erectile dysfunction and abdominal ailments in Thai and Laos.[10-14] Furthermore, it had been reported that the extract and flavone derivatives obtained from the rhizomes *Kaempferia parviflora* regulate the function of multidrug resistance associated-protein (MRP) and P-glycoprotein, in cancer cells.[15] Also, a study reported that the plant possesses anti-plasmodial, antifungal and antibacterial activities.[8] In general, plant extract is considered to be the rich resource for isolating pharmacologically important natural products.[16] The phytoconstituent present in the plant extract vary significantly depending on the extraction method as well as polarity of the solvent used for extraction.[17] Of the various solvents reported for Soxhlet extraction, chloroform is widely used for isolating non-polar compounds.[17] Notably, there are not much information available on the volatile constituent present in the chloroform extract of *Kaempferia parviflora* rhizome. Thus, the current investigation was planned to undertake the putative identification of the nonpolar

volatile constituents in chloroform extract of the rhizome of *Kaempferia parviflora* and to correlate with bioactivities mainly by evaluating antioxidant and anti-microbial/fungal activities. For phytochemical identification, gas chromatography-mass spectroscopy (GC-MS) technique was used in order to perform putative analysis of volatile and semi volatile compounds quantitatively.

## MATERIALS AND METHODS

### Chemicals and Reagents

Chloroform (EMPLURA), petroleum ether (LR Grade), potato dextrose broth, nutrient broth, potato dextrose agar medium, and dimethyl sulfoxide (DMSO) were purchased from Hi Media Laboratories, India. The rest of chemicals employed in the study were of analytical grade.

### Sample Harvesting and Authentication

The fresh rhizomes of *Kaempferia parviflora* were collected from Senapati district, Manipur, North-East India, during December, 2017. The plant authentication was done by the Botanical Survey of India, Shillong with Ref. No. BSI/ERC/Tech/19-20/726.[18]

### Preparation of Chloroform Extract

The harvested rhizomes were cleaned thoroughly with normal water followed by distilled water. The rhizomes were then sliced into thin pieces with the help of a clean sharp knife and then shade air dried for 18 days at ambient temperature. The completely dried rhizomes were ground using an electrical grinder to obtain powder form. About ~115 g of *Kaempferia parviflora* rhizome powder was taken and extracted with 300 ml each of petroleum ether and chloroform sequentially using a Soxhlet extractor at their respective boiling points till extractions were complete. The extracts collected were concentrated by removing solvents from them using a vacuum rotary evaporator at reduced pressure. The concentrated extracts were obtained as viscous semi solid masses which were then stored under refrigerated condition for further analyses.

### Phytochemical Screening

Qualitative screening of phytochemicals in the extract was performed using the standard protocol reported earlier.[19]

### Anti-microbial and Anti-fungal Activities

#### *Test pathogens*

Bacterial pathogens employed were Gram-positive bacteria, *Mycobacterium smegmatis* (MTCC 6), *Micrococcus luteus* (microbial type culture collection (MTCC 106), *Bacillus subtilis* (MTCC 121) and Gram-negative bacteria, *Escherichia coli* (MTCC 739). Fungal test strains employed were animal pathogen *Aspergillus niger* (MTCC 1344) and plant pathogen *Rhizoctonia solani* (MTCC 4633). The positive control for anti-fungal and anti-bacterial screening was represented by fluconazole (25 microgram (µg)/millilitre (ml)) and ampicillin (25 µg/ml) respectively.

### Biocidal Assays

Using agar well diffusion method.[20] (Bauer *et al.* 1966), the extract (25 µg/ml in DMSO) was screened for anti-bacterial and anti-fungal activities with a slight modification. DMSO control represented the vehicle control group.

### Antioxidant Studies

The DPPH•/ ABTS•⁻ radical scavenging assay.[19,21] (Atom *et al.* 2021 Shaikh *et al.* 2019) was performed by following the reaction between fixed concentration of DPPH• (50 µM) or ABTS•⁻ (120 µM) radicals and the increasing concentrations of extract through spectrophotometer as described previously.[19,21] The 50% inhibitory concentration ($IC_{50}$) value (concentration of the extract needed to reduce the absorbance of DPPH•/ ABTS•⁻ radicals by 50%) was calculated from the percentage scavenging activity of extract.

### Lipid Peroxidation Assay

The extract was examined for inhibition of lipid peroxidation by using soy lecithin liposomes and thiobarbituric acid (TBA) regent.[19,21]

### GC-MS Experiment

GC-MS experiment was performed on a GC instrument (Agilent Technologies 7890 A) coupled to mass spectrometer (AccuTOF GCv, JMS-T100GCV, JEOL, Japan) under the following experimental conditions as reported previously.[19] About 1 µl of the sample was injected using helium as a carrier gas with a flow rate of 1 ml/min. The analytical column attached to the GC was HP5 column and the injector temperature was set at 250°C. The MS was operated in electron ionisation (EI) mode at 70eV. The compounds were identified by comparing mass spectra with National Institute of Standard and Technology (NIST), MS library Search 2.0-[Q]. Calculation of the relative abundance of each chromatogram peak was performed by normalizing its area with the total area of chromatogram.

## RESULTS

### Phytochemical Classification

The outcomes of the initial phytochemical screening of chloroform extract of rhizomes of *Kaempferia parviflora* are provided in the Table 1. In brief, phytochemical segregation of the chloroform extract of *Kaempferia perviflora* rhizomes suggested the existence of quinones, phenolic compounds, steroids, terpenoids, carbohydrates, glycosides, coumarin, alkaloids, protein and flavonoids.

### Anti-microbial and Anti-fungal Actions

The extract was tested for anti-microbial and anti-fungal actions at a concentration of 25 µg/ml against the bacterial and fungal test pathogens as described in the method section (Figure 1 and 2). Notably the extract showed potency against all the bacterial test pathogens (Table 2). However, with regard to anti-fungal activity, the extract was effective against the plant pathogen (*R. solani*) but, not against human pathogen (*Aspergillus niger*) (Table 3). The maximum potency of the extract was seen against a bacterial pathogen *Micrococcus luteus* with the diameter of zone of inhibition of 24 ± 2 mm. The difference in the zone of inhibition

**Table 1: Preliminary phytochemical screening of the chloroform extract of the rhizomes of *Kaempferia parviflora* Wall.ex Baker.**

| Phytochemical Components | Observation |
|---|---|
| | Chloroform Extract |
| Quinones | Positive |
| Phenols | Positive |
| Terpenoids | Positive |
| Steroids | Positive |
| Carbohydrates | Positive |
| Glycosides | Positive |
| Coumarin | Positive |
| Alkaloids | Positive |
| Protein | Positive |
| Flavanoids | Positive |

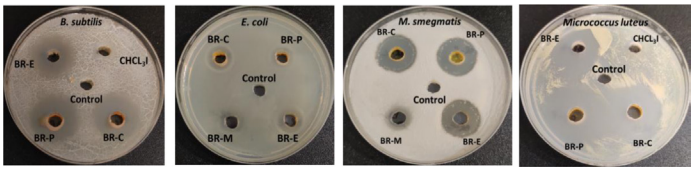



**Figure 1:** Agar plates show antibacterial activity of BR-C (chloroform extract) in terms of the zone of inhibition (mm) against pathogenic bacteria. The standard antibiotic, Ampicillin was used as a positive control. BR-C: Chloroform extract; BR-E, BR-P, BR-M, $CHCL_3I$ are excluded in the study.

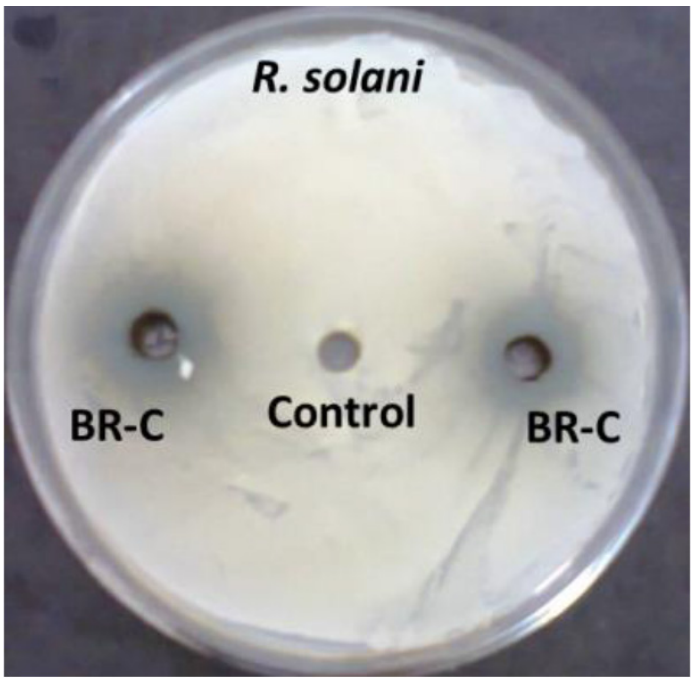



**Figure 2:** Agar plate shows antifungal activity of BR-C (chloroform extract) in terms of the zone of inhibition (mm) against plant pathogen (*R. solani*).

**Table 2: Antibacterial activity of the chloroform extract of the rhizomes of *Kaempferia parviflora* Wall.ex Baker. Values are mean of duplicate readings (mean ± SD).**

| Test Sample | Concentration (µg/ml) | Diameter of zone of inhibition (in mm) | | | |
|---|---|---|---|---|---|
| | | B S | M L | E C | M S |
| Extract | 25 | 16 ±1 | 24±2 | 14±1 | 22±1 |
| Ampicillin (Positive control) | 25 | 25±0 | 30.5±0.5 | 28±1 | 24±0 |

**Note:** B S = *Bacillus subtilis*; M L= *Micrococcus luteus*; E C = *Escherichia coli*; M S = *Mycobacterium smegmatis*

**Table 3: Antifungal activity of the chloroform extract of the rhizomes of *Kaempferia parviflora* Wall.ex Baker. Values are mean of duplicate readings (mean ± SD).**

| Test Sample | Concentration (µg/ml) | Diameter of zone of inhibition (in mm) | |
|---|---|---|---|
| | | *Rhizoctonia solani* | *Aspergillus niger* |
| Extract | 25 | 16.5±0.5 | - |
| Fluconazole (Positive control) | 25 | 20.5±0.5 | 17.5±0.5 |

against various pathogens might be due to the diffusing potency of the extract against the particular organism. These results thus suggested that the extract remarkably exhibited broad spectrum of activity, especially toward the bacteria.

## Antioxidant Activity

Antioxidant properties of the extract were evaluated in terms of radical scavenging. The $IC_{50}$ values of the extract to scavenge stable radicals like $DPPH^{\bullet}$ and $ABTS^{\bullet-}$ was 3.0 ± 0.15 mg/ml and 19.4 ± 1.2 µg/ml, respectively, shown in Figure 3. To further substantiate these results, the extract was evaluated for its ability to inhibit the process of lipid peroxidation. For this, we used an *in vitro* assay wherein the lipids present in the liposome was oxidised by radiolytically (by high energy radiation) generated hydroxyl radical from the water. Subsequently, TBA reagent reacts with oxidised lipid forming pink coloured thiobarbituric acid reactive substances (TBARS) which is measured by recording absorbance at 532 nm. The presence of antioxidant is known to inhibit the oxidation of lipid by scavenging hydroxyl radicals. In line with assumption, the presence of extract in the liposomal solution showed significant decrease of lipid peroxidation as evidenced by the decrease in TBARS levels in the extract treated group as compared to control. The percentage decrease of lipid peroxidation at 20 µg/mL of extract was observed as 80.0 ± 4.5%.

## Chemical Composition

GC-MS analysis provided the chemical profiling of chloroform extract of the rhizome and suggested the chemo type of the plant sample which are shown in Figure 4 and 5. Retention time (RT), molecular weight (MW) and molecular formula of the non-polar volatile compounds present in the extract are provided in Table 4. Altogether, twenty-five compounds have been identified in the extract belonging to different classes such as alcohol, monoterpene, amine, hydrocarbon, phenolic, heterocyclic, amino acid, sesquiterpene, aldehyde, steroid, and sterol among others. Of these 5,7-dimethoxy-2-phenyl 4H-1-benzopyran-4-one and hydroxyurea showed the maximum and minimum relative

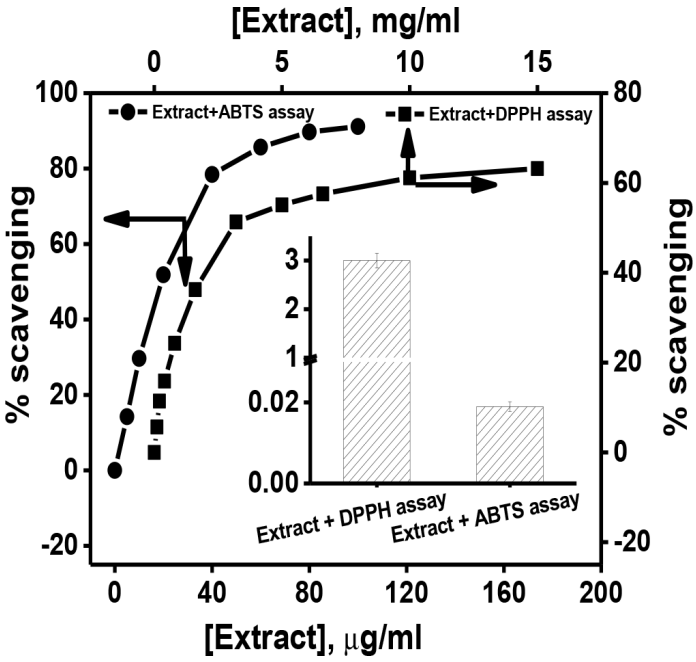



**Figure 3:** The graph of % scavenging of DPPH and ABTS radical vs concentration of extract. Inset shows the $IC_{50}$ values of extract obtained from DPPH and ABTS assays. Values are mean of duplicate readings (mean ± SD).

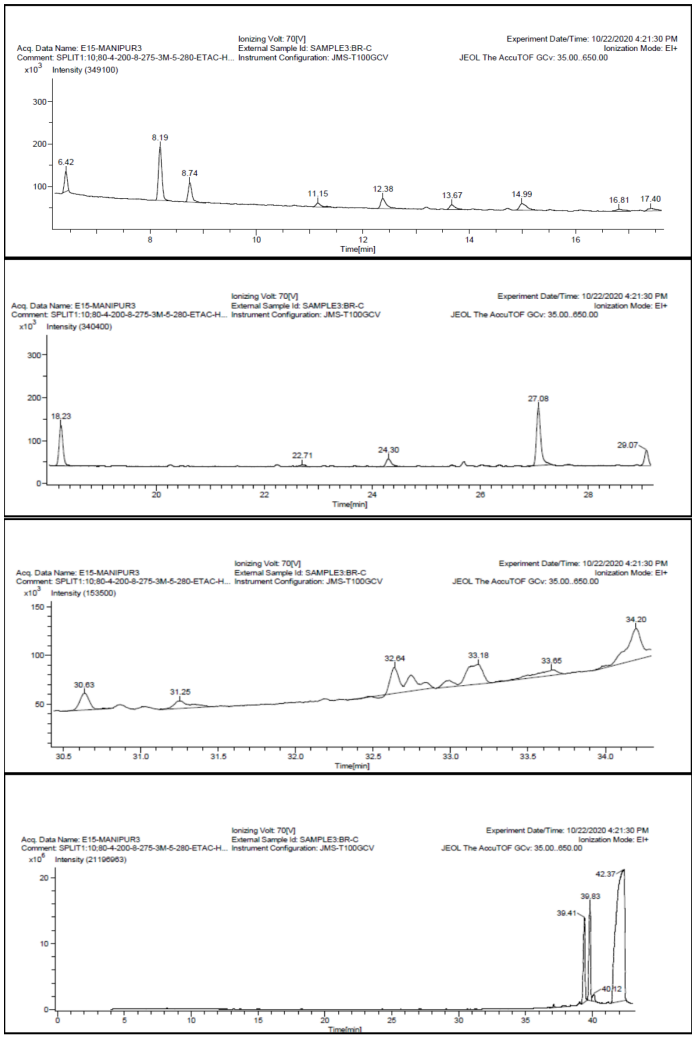



**Figure 4:** GC-MS chromatograms show twenty-five peaks of the chloroform extract of Kaempferia parviflora Wall.ex Baker Rhizomes.

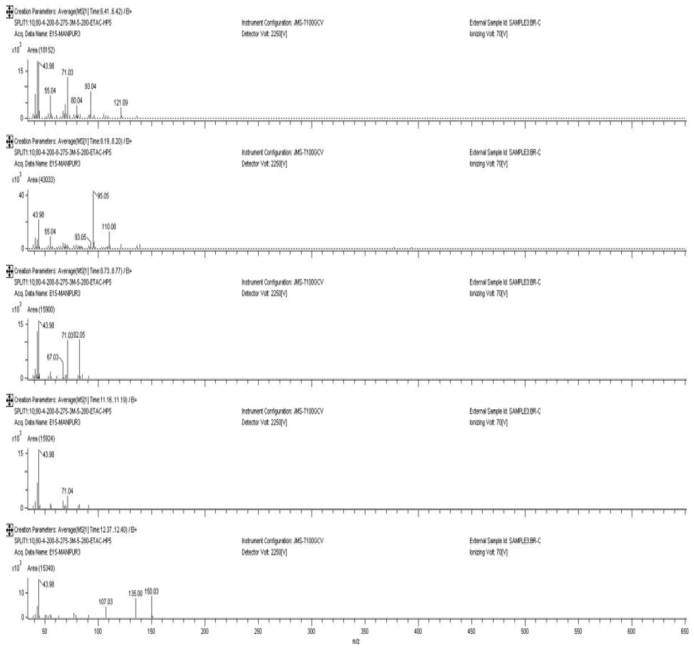


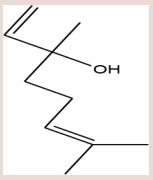
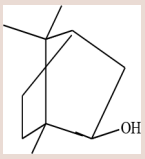
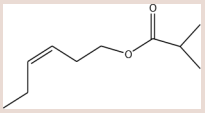
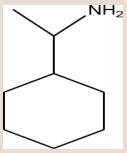
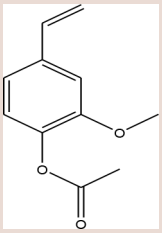
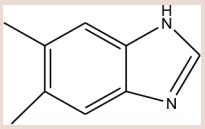
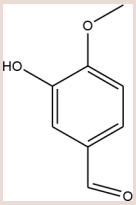
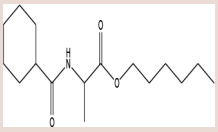
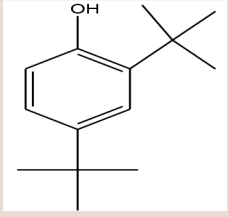
**Figure 5:** Mass fragment peaks of the representative retention times 6.41 min, 8.19 min 8.73 min,11.16min and 12.37 min of chloroform extract of Kaempferia parviflora Wall.ex Baker Rhizomes.

abundance of 76.4% and 0.002% respectively. Notably, twelve out of twenty-five compounds have been well reported for their medicinal properties (Table 4). For example, compounds like 3,7-dimethyl 1,6-Octadien-3-ol and isoborneol possess anti-inflammatory/ anti-cancer and antimicrobial properties respectively.[22,23] Similarly, 2,4-bis[1,1dimethylethyl] phenol reported to possess the properties such as antioxidant, anticancer, antifungal, antibacterial and protection against trimethyltin (TMT)-induced cognitive dysfunction.[9,11,24] Hydroxyurea possesses anti-cancer activity, ledene oxide-(l) shows anti-tumor, analgesic, antibacterial, anti-inflammatory sedative and fungicidal properties.[25] Diazoprogesterone is reported to show anti-HIV property and stigmasterol mediates antiosteoarthritic, antioxidant, antihypercholestrolemic, antitumor, hypoglycaemic, antimutagenic and anti-inflammatory effects.[26] γ-Sitosterol is reported for cytotoxic activity against colon and liver cancer cell lines. Keeping in view of the above-mentioned pharmacological activities, it is quite clear that the rhizome extract of *Kaempferia perviflora* is rich in bioactive compounds and this explains the ethanomedicinal importance of the plant.

## DISCUSSION

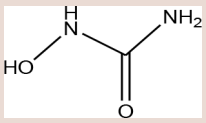
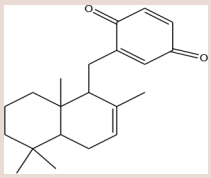
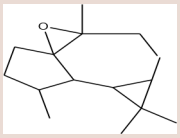
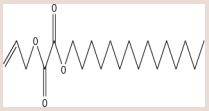
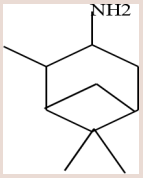
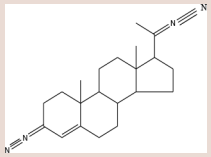
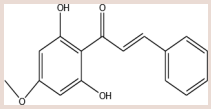
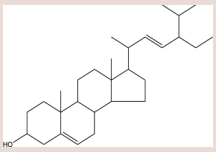
There are several reports available in the literature, wherein the extract of *Kaempferia parviflora* rhizome have been evaluated for various biological activities. For example, methanolic extract of *Kaempferia parviflora* rhizome has shown antimicrobial activities against a wide range of pathogenic bacterial species which includes *Bacillus cereus, Shigella dysenteriae, Staphylococcus aureus, Streptococcus faecalis, Salmonella typhi, Proteus vulgaris, Candida tropicalis* and *Saccharomyces cerevisiae.*[27,28] Interestingly, the antimicrobial activity of the extract was due to the presence of phytochemicals such as flavonoids and tannins in this study.[27,28] In additional study, methanolic extract of *Kaempferia parviflora* is shown to exhibit potent antioxidant activity by the radical-scavenging DPPH assay with $IC_{50}$ of ~61.5 mg/ml. In the similar way, ethanol/water extract of *Kaempferia parviflora* rhizomes is reported for anti-fungal activity against *Trichophyton rubrum, Trichophyton mentagrophytes and Microsporum gypseum,*[29] anti-biofilm activity against *Streptococcus mutans*[30] and antiviral activity against H5N1 avian influenza.[31] Further, volatile oils obtained from the *Kaempferia parviflora* have been reported to have antibacterial activity against *S. aureus* and *Pseudomonas aeruginosa* and ethyl acetate extract against *Helicobacter pylori.*[3,32] With regard to phytochemical composition, a recent study has reported the presence of lipophenol, alkaloids, glycosides, phenol, saponin and tannin in the water extract and flavonoid, polyphenol, terpene and steroid in the ethanol extract of the rhizome of *Kaempferia parviflora* Wall. ex Baker.[33] Another study reported that volatile oils obtained from the dried rhizomes of *Kaempferia parviflora* consist of at least 20 compounds. Out of them, α-copaene (11.68%), dauca-5, 8-diene (11.17%), camphene (8.73%), β-pinene (7.18%), borneol (7.05%), and linalool (6.58%) have been observed as major components.[34] Another similar study on phytochemical profiling of volatile oil obtained from the hexane extract of *Kaempferia parviflora* rhizomes has reported borneol (10.24%), β-pinene (8.60%), camphene (7.62%), α-copaene (7.23%) and linalool (6.40%) as the dominant components. Further, there are also few reports on the identification and quantitation of flavonoid constituents in *Kaempferia parviflora.*[14,35] The existence of 11 flavonoid constituents have been quantified on *Kaempferia parviflora* rhizome using gas chromatographic method in which 5,7,4′ trimethoxyflavone and 5,7 dimethoxyflavone were considered to be main constituents.[35]

**Table 4: List of twenty-five bioactive compounds identified through GC-MS evaluation of the chloroform extract of *Kaempferia parviflora* Wall.ex Baker rhizome.**

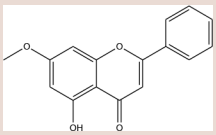
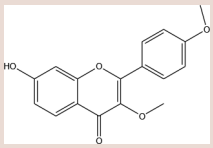
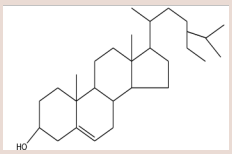
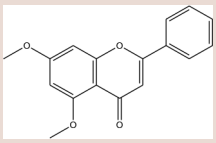
| Peak No. | RT (Min) | Area [intense*sec] | Molecular formula | Mol. Wt.(MW) | Relative abundance | Compound Name | Structure | Probability % |
|---|---|---|---|---|---|---|---|---|
| 1 | 6.42 | 166991.40 | $C_{10}H_{18}O$ | 154 | 0.014 | 3,7-dimethyl 1,6-Octadien-3-ol |  | 53.4 |
| 2 | 8.19 | 565707.15 | $C_{10}H_{18}O$ | 154 | 0.050 | Isobomeol |  | 25.3 |
| 3 | 8.73 | 224864.45 | $C_{10}H_{18}O_2$ | 170 | 0.020 | Cis-3-Hexenyl iso-butyrate |  | 25.3 |
| 4 | 11.16 | 63748.32 | $C_8H_{17}N$ | 127 | 0.005 | R-()-Cyclohexylethyamine |  | 70.3 |
| 5 | 12.37 | 174625.63 | $C_{11}H_{12}O_3$ | 192 | 0.015 | 4-Acetoxy-3-methoxystyrene |  | 55.5 |
| 6 | 13.65 | 77342.14 | $C_9H_{10}N_2$ | 146 | 0.006 | 5,6-dimethyl 1H-Benzimidazole |  | 56.2 |
| 7 | 14.98 | 149805.38 | $C_8H_8O_3$ | 152 | 0.013 | Benzaldehyde,3-hydroxy-4-methoxy |  | 16.7 |
| 8 | 17.41 | 67790.46 | $C_{16}H_{29}NO_3$ | 283 | 0.006 | I-Alanine,N-(cyclohexylcarbonyl) hexyl ester |  | 11.6 |
| 9 | 18.23 | 461034.03 | $C_{14}H_{22}O$ | 206 | 0.041 | 2,4-bis[1,1-dimethylethyl]Phenol |  | 44.1 |

continued...

**Table 4: List of twenty-five bioactive compounds identified through GC-MS evaluation of the chloroform extract of *Kaempferia parviflora* Wall.ex Baker rhizome.**

| Peak No. | RT (Min) | Area [intense*sec] | Molecular formula | Mol. Wt.(MW) | Relative abundance | Compound Name | Structure | Probability % |
|---|---|---|---|---|---|---|---|---|
| 10 | 22.71 | 25448.30 | $CH_4N_2O_2$ | 76 | 0.002 | Hydroxyurea |  | 23.3 |
| 11 | 24.30 | 126586.94 | $C_{21}H_{28}O_2$ | 312 | 0.011 | 1,4-dione,2-[(1,4,4a,5,6,7,8,8a-octahydro-2,5,5,8a-tetramethyl-1-naphthalenyl)methyl,[1R-[1α,4aβ,8aα)] 2,5-Cyclohexadiene |  | 11.3 |
| 12 | 27.07 | 756182.18 | $C_{15}H_{24}O$ | 220 | 0.067 | Ledene oxide-(l) |  | 15.4 |
| 13 | 29.07 | 177238.42 | $C_{11}H_{22}O_2$ | 186 | 0.015 | Methyl 8-methyl-nonanoate |  | 32.7 |
| 14 | 30.63 | 73981.29 | $C_{20}H_{36}O_4$ | 340 | 0.006 | Oxalic acid,allyl pentadecyl ester |  | 7.15 |
| 15 | 31.26 | 57815.82 | $C_{10}H_{19}N$ | 153 | 0.005 | 2,6,6-Trimethyl-bicyclo[3,1,1]hept-3-ylamine |  | 13.2 |
| 16 | 32.63 | 192249.30 | $C_{16}H_{26}O$ | 234 | 0.017 | Cis,cis,cis-7,10,13-Hexadecatrienal |  | 14.1 |
| 17 | 33.17 | 180535.31 | $C_{19}H_{30}O_2$ | 290 | 0.016 | 13,16-Octadecadiynoic acid,methy ester |  | 38.6 |
| 18 | 33.66 | 61938.97 | $C_{21}H_{30}N_4$ | 338 | 0.005 | Diazoprogesterone |  | 29.5 |
| 19 | 34.20 | 238494.12 | $C_{22}H_{40}O_2$ | 336 | 0.021 | 2-[7-heptadecynyloxy]tetrahydro 2H-Pyran |  | 34.8 |
| 20 | 37.11 | 3060270.74 | $C_{16}H_{14}O_4$ | 270 | 0.272 | 1-[2,6-dihydroxy-4-methoxyphenyl]-3-phenyl,(E)2-Propen-1-one |  | 85.0 |
| 21 | 37.94 | 3870160.67 | $C_{29}H_{48}O$ | 412 | 0.345 | Stigmasterol |  | 36.1 |

continued...

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 22 | 39.40 | 118088777.86 | $C_{16}H_{12}O_4$ | 268 | 10.533 | 5-hydroxy-7-methoxy-2-phenyl 4H-1-Benzopyran-4-one |  | 92.7 |
| 23 | 39.82 | 122557084.24 | $C_{17}H_{14}O_5$ | 298 | 10.931 | 7-Hydroxy-3-methoxy-2-p-methoxyphenyl-4H-chromen-4-one |  | 49.1 |
| 24 | 40.12 | 12510587.68 | $C_{29}H_{50}O$ | 414 | 1.115 | Y-Sitosterol |  | 39.3 |
| 25 | 42.37 | 857142724.33 | $C_{17}H_{14}O_4$ | 282 | 76.454 | 5,7-dimethoxy-2-phenyl 4H-1-Benzopyran-4-0ne |  | 88.0 |

Furthermore, high-performance liquid chromatography (HPLC) analysis of methoxyflavones in *Kaempferia parviflora* ethanolic extract revealed 5,7,4′ trimethoxyflavone, 5,7 dimethoxyflavone, and 3,5,7,3′,4′ pentamethoxyflavone as major components.[36,37] However, to the best of our knowledge, there are no reports on the putative identification of phytoconstituents present in the chloroform extract of *Kaempferia parviflora* Wall ex Baker rhizome and its evaluation for antioxidant/antimicrobial/antifungal activities. In general, dissolution of bioactive compounds present in the plant sample is higher with higher polarity of the solvent and thus plant extracts prepared using polar solvents like water, and ethanol contain generally a large number of bioactive compounds with close retention factor (R*f*) values. This causes isolation of bioactive compounds from polar extracts a quite challenging task.[16,17] Accordingly, to overcome this issue, here in present study, the sequential chloroform extraction method was followed to prepare extract from rhizome of *Kaempferia parviflora* Wall ex Baker. The extract prepared so was used for GS/MS analysis and the evaluation of various bioactivities. The GC-MS analysis revealed the presence of several compounds with known bioactivities including antioxidant /antimicrobial / antifungal / anticancer actions. However, the novel outcome of the present investigation is the identification of a major compound, 5,7-dimethoxy-2-phenyl 4H-1-benzopyran-4-one with about 76.4% relative abundancy. This phytochemical belongs to the class of heterocyclic compounds and has not explored for any bioactivity till date. So, future studies should focus on isolating this phytochemical using chloroform extract of *Kaempferia parviflora* rhizome and understating its bioactivities (if any).

## CONCLUSION

The current study reveals that chloroform extract of *Kaempferia parviflora* rhizome is a rich source of volatile bioactive compounds which could be responsible for antioxidant and anti-microbial properties. Further, the identification of 5,7-dimethoxy-2-phenyl 4H-1-benzopyran-4-one as a major compound opens the scope for the isolation and development of novel phytochemicals/natural products from this plant for therapeutic application.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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