

## Alkaloids and non-alkaloids identified from *Crinum defixum* Ker-Gawler medicinal plant leaves

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**Abstract:** The aim of the present study was to investigate the alkaloid and non-alkaloid compounds present in the *Crinum defixum* Ker-Gawler medicinal plant leaves. The plant was extracted for various solvents in increasing order of polarity from using n-hexane, chloroform, ethyl acetate, acetone, ethanol, butanol and methanol. The preliminary phytochemical analysis of various extracts of *Crinum defixum* Ker-Gawler leaves contains many bioactive chemicals like alkaloids, flavonoids, saponins, terpenoids, amino acids and phenolic compounds are qualitatively analyzed. Thus, the present study was performed to investigate the separation, identification of various classes of phytocomponents present in the various fractions of *C.defixum* Ker-Gawler leaves using FT-IR, GC-MS, NMR and MASS Spectral techniques.

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**Keywords:** *Crinum defixum* Ker-Gawler, Phytochemical screening, solvent extraction, Alkaloids, non-alkaloids.

### 1. Introduction

The plants of the genus *Crinum* (Amaryllidaceae) has used in Asian folk and traditional medicine as rubefacient, tonic and for treatment of allergic disorders and tumor diseases [1]. The genus of *Crinum* (Amaryllidaceae) is a big family and contains about 160 species, many of which have vanished [2]. Most of the *Crinum* species are common emetic, laxative, expectorant, diaphoretic, anti-asthmatic, analgesic, anti-inflammatory, anti-microbial and anti-tumor remedies in various folkloric medicines [3]. Already the *Crinum* genus has yielded more than 170 different compounds, most of which are alkaloids. These have shown significant analgesic, antitumor and antiviral activities [4]. Phytochemical investigations have resulted in isolation of several classes of compounds and have been focused predominantly on alkaloids whereas the non-alkaloid constituents are much less attention [5].

*C.defixum* Ker-Gawler (Amaryllidaceae) has abundantly growing on rivers and canals side's in wet conditions. The *C.defixum* has commercial, economical and medicinal importance. The *C.defixum* Ker-Gawler is one of the *Crinum* genuses [6]. It is commonly known as Bon-naharu (Meaning wild garlic) and this plant genus having lot of medicinal activities. The leave extracts are used to treat pimples, body-ache, leprosy, paronychia itching, and otitis. The crushed bulbs are used to treating nauseant, emetic, emollient, diaphoretic, burns, whitlow and carbuncle [7]. The bulbs of this plant is fusiform, flowers are sessile, fragment at night and tinged with red [8]. The *C.defixum* is reported to contain the active constituents such as caranine, crinamine, crinine, galanthamine,

galanthine, haemanthamine and hippestrine. The new alkaloid 5  $\alpha$ -hydroxyhomolycorine has also been reported in the recent years [9]. The ethanol and methanol extracts of the *C.defixum* Ker-Gawler have been reported to free radical scavenging, antianalgesic and antigenotoxic properties [10]. The main focus of this study was separation, identification of various classes of phytocomponents present in the various fractions of *C.defixum* Ker-Gawler leaves using FT-IR, GC-MS, NMR and MASS Spectral techniques.

### 2. Materials And Methods

#### 2.1. Collection Of Plant Materials

The leaves of *C.defixum* Ker - Gawler were collected from Poondi village, Thanjavur District, Tamilnadu. The botanical identity (Voucher No: A.A.R 003 on 04-02-2013) of the plant of was confirmed by Dr. S.John Britto, Rapinat Herbarium, St. Joseph's College, Tiruchirappalli.

#### 2.2. Preparation Of Extracts

The fine powder (5 kg) was extracted with 95% ethanol at room temperature for ten days. The extract were filtered and concentrated under reduced pressure in a rotary evaporator and extracted for various solvents in increasing order of polarity from using n-hexane, chloroform, ethyl acetate, acetone, ethanol, butanol and methanol. After that the extract was taken in a beaker and kept in a water bath and heated at 30-40 °C till all the solvent got evaporated. All the extracts were tested for the presence bioactive compounds by using standard methods. The dried extracts were subjected to preliminary phytochemicals and also confirmed by spectral analysis.

### 2.3. Phytochemical Screening

The preliminary phytochemical analysis of various extract of *C.defixum* Ker-Gawler plant leaves revealed the following phytochemicals (Table 1).

**Table 1. Preliminary phytochemical constituents of *C.defixum* Ker-Gawler leaves.**

S.N	Phytochemicals	Hexane Extract	Chloroform Extract	Ethyl acetate Extract	Acetone Extract	Ethanol Extract	Butanol Extract	Methanol Extract
1.	Alkaloids	-	Present	Present	Present	Present	-	-
2.	Flavonoids	-	Present	-	-	-	Present	Present
3.	Terpenoids	Present	Present	Present	-	-	-	-
4.	Glycosides	-	-	-	-	-	-	-
5.	Saponins	-	Present	Present	Present	Present	-	-
6.	Steroids	Present	-	-	Present	-	-	-
7.	Carbohydrates	-	-	-	-	-	-	-
8.	Phenolic Compounds	Present	Present	Present	Present	Present	Present	-
9.	Tannins	-	-	-	-	-	-	-
10	Amino acids	Present	-	-	Present	Present	Present	Present

### 3. Analysis of Phytochemicals

#### 3.1. GC-MS analysis

Chromatography is the term used to describe a separation technique in which a mobile phase carrying a mixture is caused to move in contact with a selective absorbent stationary phase. Gas chromatography coupled with mass spectrometer is one of the most widely used techniques. The analysis was simple, sensitive and effective in separating components of mixtures. Identification of phytoconstituents present in the extract concentrate of the plants can be achieved through GC-MS.

#### 3.2. FT-IR analysis

FT-IR (Fourier Transform- Infra red) spectra were obtained using Perkin Elmer FT-IR 450-4000 IN

KBr disc and absorption peaks in terms of wave numbers ( $\text{cm}^{-1}$ ).

#### 3.3. NMR analysis

NMR (Nuclear Magnetic Resonance) was acquired on Bruker at 300 and 400 MHz ( $^1\text{H}$ ) and 100 MHz ( $^{13}\text{C}$ ). Chemical shifts were recorded as  $\delta$  value (ppm) DMSO,  $\text{D}_2\text{O}$  and Chloroform using as an inert solvent. Perkin Elmer ICP-OES analysis was used for sodium and potassium.

Hence in the current investigation, identification of secondary metabolites of hexane, chloroform, ethyl acetate, acetone, ethanol, butanol and methanol extract of *Crinum defixum* Ker-Gawler leaves.

**Table 2: A list of alkaloids and non-alkaloids isolated from *Crinum defixum* Ker-Gawler leaves.**

S.N	Name of the phytochemicals	Solvent extraction	M.F	M.W	Spectral data	Activity
<b>Alkaloids</b>						
1.	Piperazine, 1,4-bis (2-methoxybenzoyl)-	Ethanol	$\text{C}_{20}\text{H}_{22}\text{N}_2\text{O}_4$	354.39	GC-MS	Unknown alkaloid
2.	Piperazine-1-carboxylic acid, 4-(2-fluoro-4-(1-oxopropyl) phenyl)-, ethyl ester	Methanol	-	-	GC-MS	Unknown alkaloid
3.	Ethanone, 1-(2,5-diphenyl-2H-1,2,3-triazol-4-yl 3-oxide)-, oxime	Acetone	-	-	GC-MS	Unknown alkaloid
4.	7-(5-Cyclohexylpentyl) - 6-hydroxy-5,8-quinolinedione	Acetone	-	-	GC-MS	Unknown alkaloid

<b>Flavonoids</b>						
5.	4',5,7-Trihydroxy isoflavone (Genistein)	Chloroform, ethyl acetate, butanol and methanol	C <sub>15</sub> H <sub>12</sub> O <sub>5</sub>	272	FT-IR, GC-MS, <sup>1</sup> H, <sup>13</sup> C-NMR and MS	Antitumor agent, antioxidant, antiangiogenic and immuno-suppressive activities.
6	4H-1-Benzopyran-4-one, 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy (Quercetin)	Ethyl acetate	C <sub>15</sub> H <sub>10</sub> O <sub>7</sub>	302.236	GC-MS	Prevention and treatment of cancer.
<b>Steroids</b>						
6.	Estra-1,3,5(10)-trien-17a'-ol	n-Hexane and Acetone	C <sub>19</sub> H <sub>26</sub> O <sub>2</sub>	286.40	GC-MS	Androgenic- alopecia (Hairloss) activity.
<b>Alkane</b>						
7.	Cyclodocosane, ethyl-	Methanol	C <sub>24</sub> H <sub>48</sub>	336.63792	GC-MS	No activity reported.
<b>Alkene</b>						
8.	3-Eicosene, (E)-	Methanol	C <sub>20</sub> H <sub>40</sub>	280.5316	GC-MS	No activity reported.
9.	3- Octadecene, (E)-	Methanol	C <sub>18</sub> H <sub>36</sub>	252.4784	GC-MS	No activity reported.
10.	9-Hexacosene	Methanol	C <sub>26</sub> H <sub>52</sub>	364.6911	GC-MS	Analgesic, antiinflammatory activities.
<b>Acids and Esters</b>						
11.	Tridecanoic acid, 12-methyl-, methyl Ester	n-Hexane	C <sub>15</sub> H <sub>30</sub> O <sub>2</sub>	242.3975	GC-MS	Antifungal, Antibacterial activities.
12.	Tetradecanoic acid, 12-methyl-, methyl ester	n-Hexane	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256.4241	GC-MS	No activity reported.
13.	Pentadecanoic acid, 14-methyl-, methyl ester	n-Hexane	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270.4507	GC-MS	Antioxidant, Antifungal, Antimicrobial activities.
14.	10-Octadecenoic acid, methyl ester	n-Hexane	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296.4879	GC-MS	Antioxidant, Antimicrobial activities.
15.	Heptadecanoic acid, 16-methyl-, methyl Ester	n-Hexane	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	298.5038	GC-MS	Used against skin cancer Protein.
16.	Octadec-9-enoic Acid	n-Hexane	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282.46136	GC-MS	Antimicrobial, anti-oxidant, Cancer preventive, Anemiagenic, Anti-androgenic activities.
17.	11-Eicosenoic acid, methyl ester	n-Hexane	C <sub>21</sub> H <sub>40</sub> O <sub>2</sub>	324.5411	GC-MS	Antioxidant, Pesticide, Nematicide activities.
18.	Eicosanoic acid, methyl ester	n-Hexane	C <sub>21</sub> H <sub>42</sub> O <sub>2</sub>	326.5570	GC-MS	Alpha-glucosidase Inhibitors activities.
19.	Docosanoic acid, methyl ester	n-Hexane	C <sub>23</sub> H <sub>46</sub> O <sub>2</sub>	354.6101	GC-MS	Therapeutic, Diagnostic activities.
20.	Methyl tetra Decanoate	Chloroform	C <sub>15</sub> H <sub>30</sub> O <sub>2</sub>	242.3975	GC-MS	Antioxidant, Cancer-preventive, Hypercholesterolemic, Nematicide activities.

21.	9-Octadecenoic acid(Z)-, methyl ester	Chloroform	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296	GC-MS	Anti-inflammatory, Antiandrogenic, Cancer preventive, Dermatitigenic, Hypocholesterolemic, 5-Alpha reductase inhibitor, Anemiagenic, Insectifuge activities.
22.	pentadecanoic acid, methyl ester	Ethyl acetate	-	-	GC-MS	Unknown
23.	9, 12, 15-Octadecatrienoic acid, methyl ester,(Z,Z,Z)-,	Ethyl acetate	-	-	GC-MS	Unknown
24.	Dodecanoic acid, ethyl ester	Acetone	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	228.3709	GC-MS	No activity reported.
25.	Hexadecanoic acid, ethyl ester	Acetone	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284	GC-MS	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor activities.
26.	(E)-9-Octadecenoic acid ethyl ester	Acetone	C <sub>20</sub> H <sub>38</sub> O <sub>2</sub>	310	GC-MS	Antioxidant, anti-inflammatory activities.
27.	Pentadecanoic acid, 14-methyl-, methyl ester	Ethanol	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270.4507	GC-MS	Antioxidant, Antifungal, Antimicrobial activities.
28.	n-Hexadecanoic acid	Ethanol	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256.4241	GC-MS	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic, 5-Alpha reductase inhibitor activities.
29.	10-Octadecenoic acid, methyl ester	Ethanol	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296.4879	GC-MS	Antioxidant, Anti-microbial activities.
30.	Octadecanoic acid, methyl ester	Ethanol	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	298.50382	GC-MS	Potent Antifungal, Anti-microbial activities.
31.	9,15-Octadecadienoic acid, methyl ester,(Z,Z)-	Ethanol	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294.47206	GC-MS	No activity reported.
32.	Oleic Acid	Ethanol	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282.47	GC-MS	Antibacterial, Cancer preventive, Anemiagenic, Insectifuge, Anti-androgenic, Dermatitigenic activities.
33.	Octadecanoic acid, 3-oxo-, methyl ester	Ethanol	C <sub>19</sub> H <sub>36</sub> O <sub>3</sub>	312.49	GC-MS	No activity reported.
34.	9-Hexadecenoic	Butanol	C <sub>17</sub> H <sub>32</sub> O <sub>2</sub>	268.4348	GC-MS	No activity reported.

	acid, methyl ester, (Z)-					
35.	8-Octadecenoic acid, methyl ester	Butanol	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296.49	GC-MS	Antioxidant, Anti-microbial activities.
36.	Dodecanoic acid, methyl ester	Methanol	C <sub>13</sub> H <sub>26</sub> O <sub>2</sub>	214.3443	GC-MS	No activity reported.
37.	9-Hexa decenoic acid, methyl ester, (Z)-	Methanol	-	-	GC-MS	Unknown.
38.	9-Octadecenoic acid, methyl ester,(E)-	Methanol	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296	GC-MS	No activity reported.
<b>Others</b>						
39.	4H-1-Benzopyran-4-one, 5,7-dihydroxy-3-phenyl-	Chloroform and butanol	-	-	GC-MS	Unknown
40.	Ethyl 5,8,11,14,17-icosapentaenoate	Butanol	-	-	GC-MS	Unknown
41.	Methyl eicosa-5,8,11,14,17-pentaenoate	Methanol	-	-	GC-MS	Unknown

Activity Source\*\*: Dr. Duke's Phytochemical and Ethno botanical Databases, NCBI-Pubmed, ChemSpider (Royal Society of Chemistry) and other available literatures.

#### 4. Alkaloids isolated from *Crinum defixum* Ker-Gawler leaves.

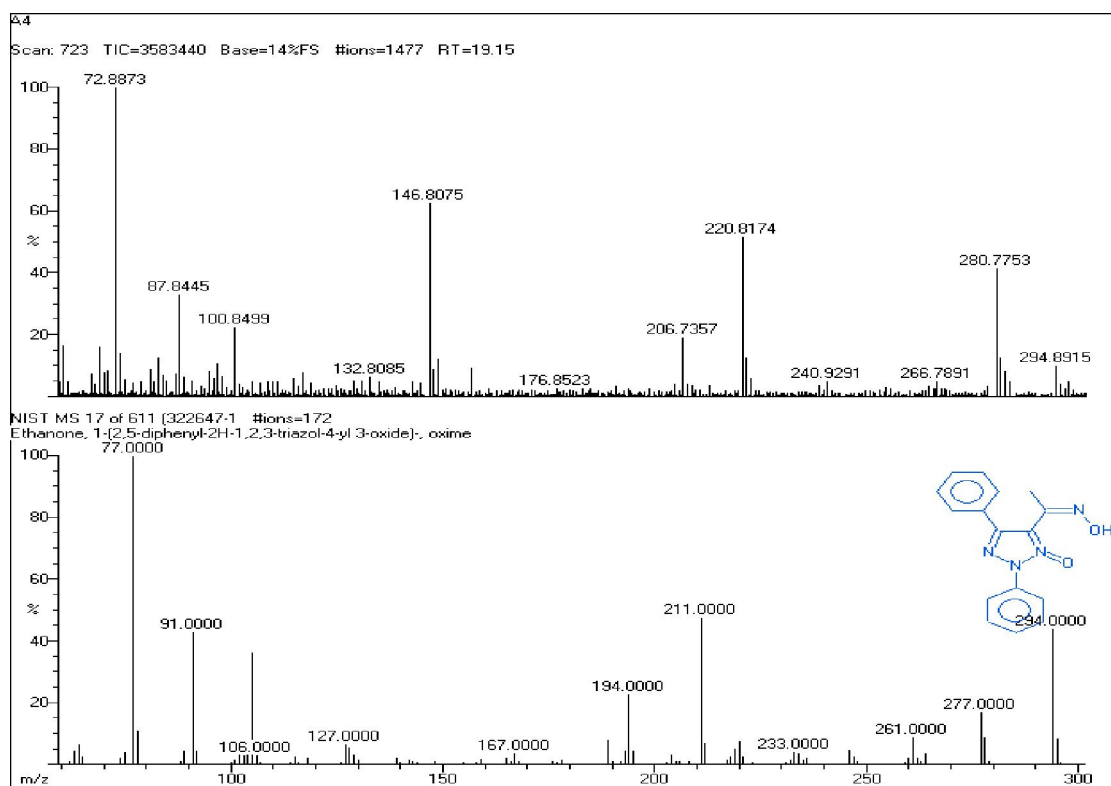
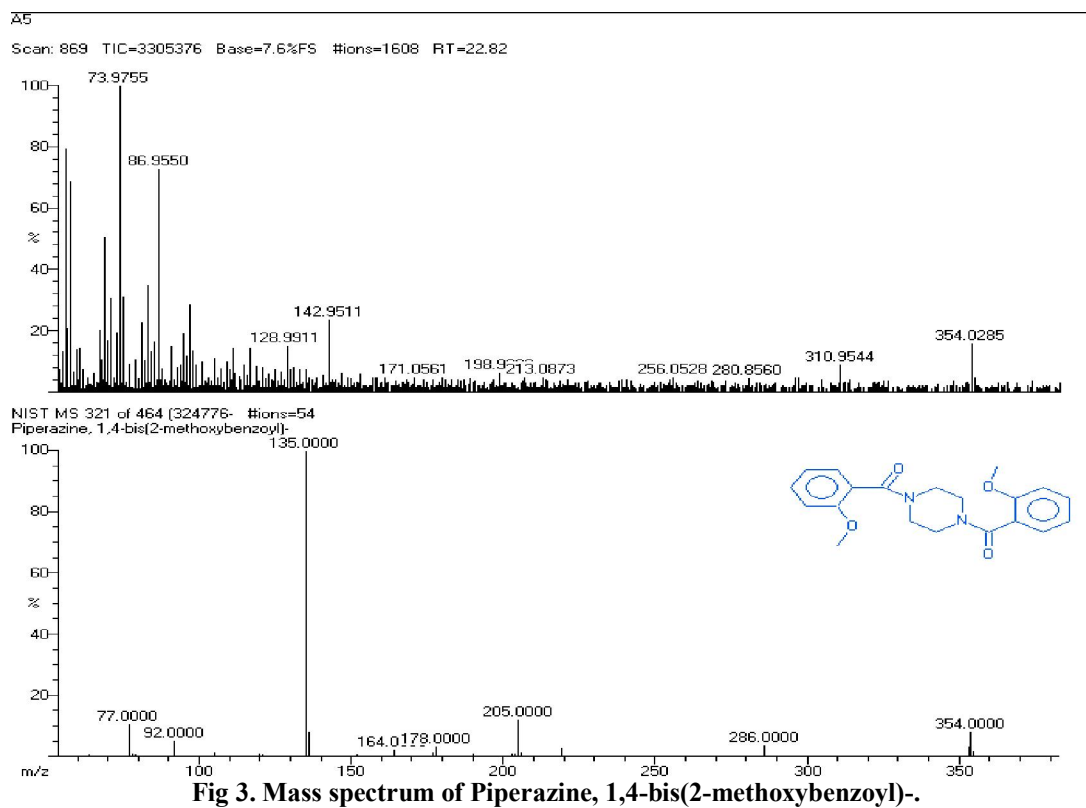
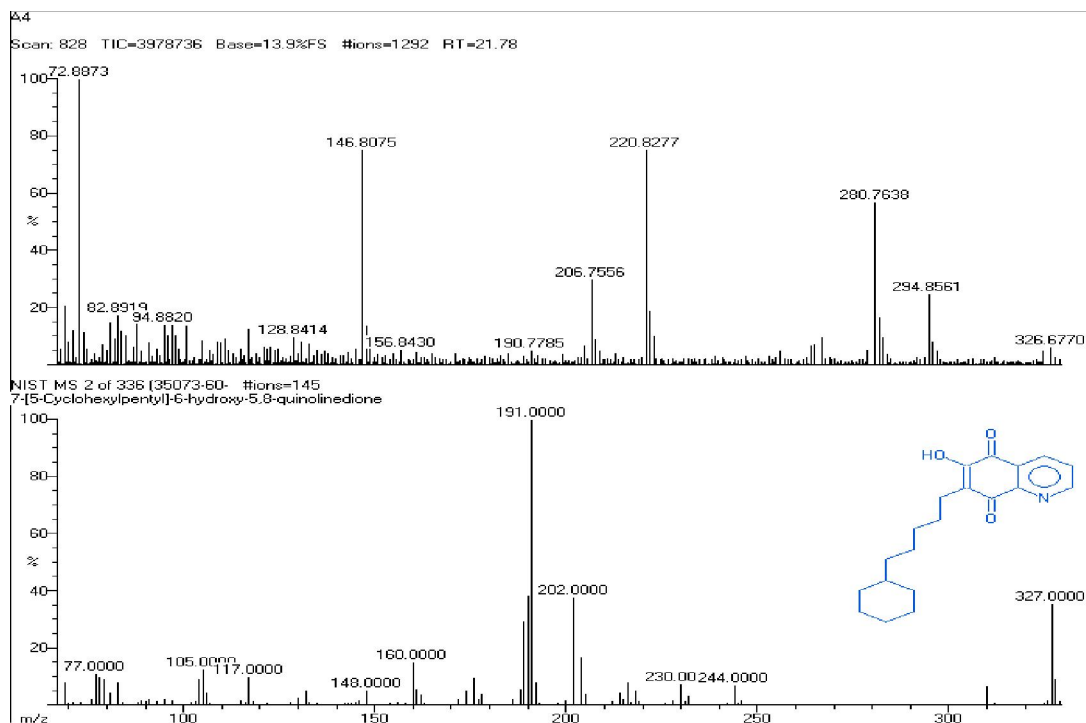


Fig 1. Mass spectrum of Ethanone,1-(2,5 diphenyl-2H-1,2,3-triazol-4-yl 3-oxide)-,oxime.



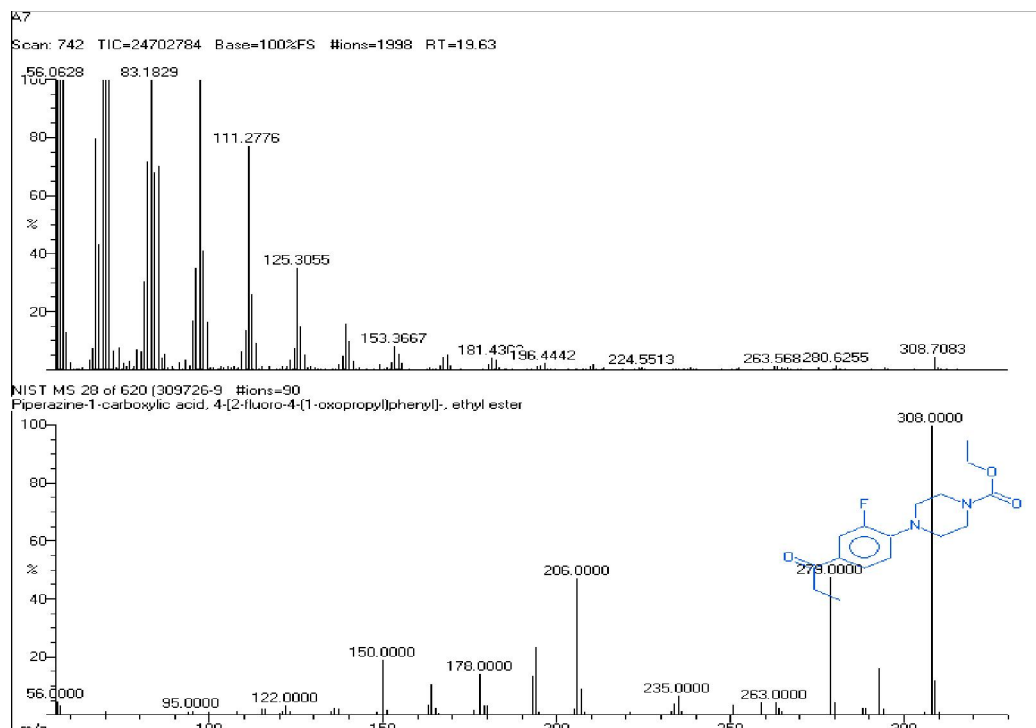


Fig 4. Mass spectrum of Piperazine-1-carboxylic acid, 4-(2-fluoro-4-(1-oxopropyl)phenyl)-, ethyl ester

#### 5. Flavonoids isolated from *Crinum defixum* Ker-Gawler leaves.

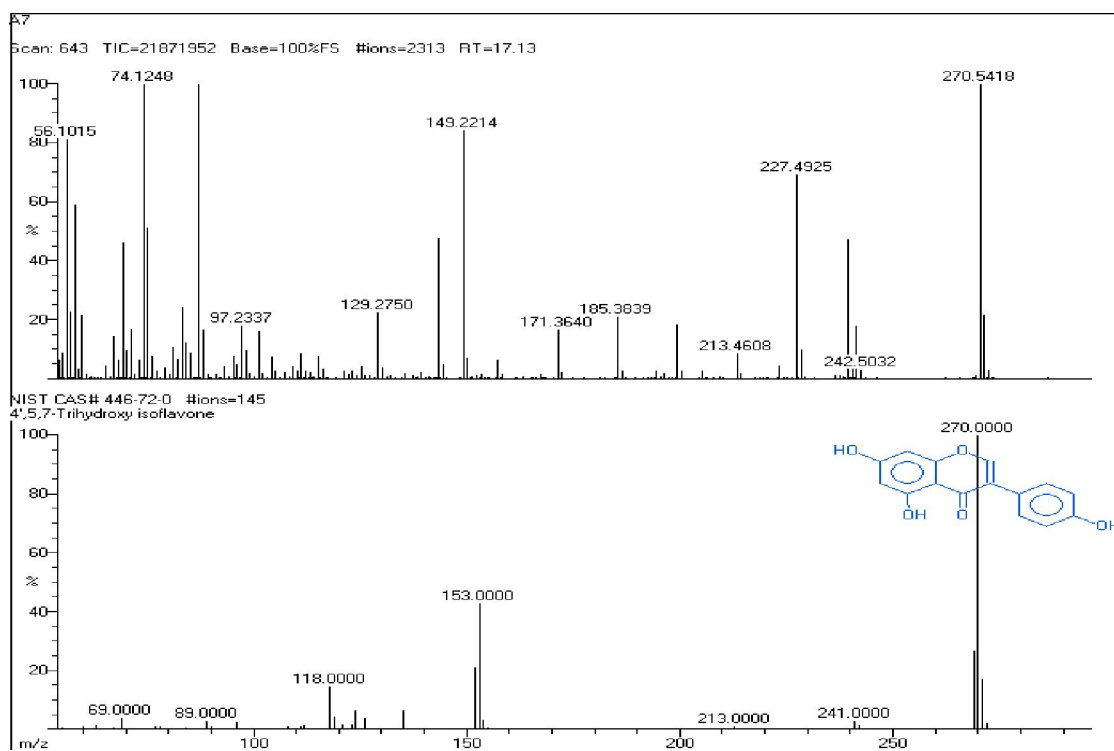


Fig 5. GC-MS- Spectrum of 4', 5, 7,-trihydroxy isoflavone

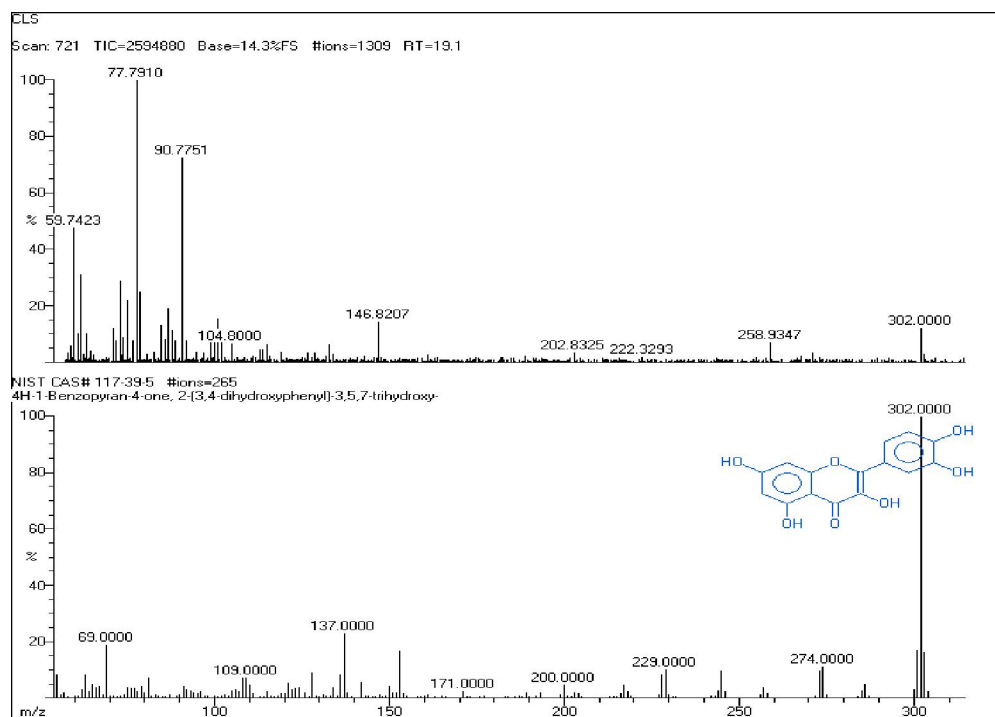


Fig 6. GC-MS- Spectrum of 4H-1-Benzopyran-4-one, 2-(3, 4-dihydroxyphenyl)-3,5,7-trihydroxy

## 6. Result and Discussion

N.Thi Ngoc Tram *et al* (2002)[20], reported that (-) Galanthamine, (+) Haemanthamine (3-Epicrinamine, Hemanthamine), Hipeastrine, (-) Lycorine (Narcissine, Galanthidine) types of alkaloids are reported that the after 1985. Recently a new alkaloid 5 $\alpha$ -hydroxy homolycorine has also been isolated from *Crinum defixum* Ker-Gawler bulbs and M.Bordoloi *et al*,(2009) reported that (E)-N' -[(E)-2-butenoylhydrazide] has been isolated from *Crinum defixum* Ker-Gawler root bulb. This Hydrazide compound imparted a clear dose dependent protective effect against the genotoxic effect of H<sub>2</sub>O<sub>2</sub> with those reported in the previous literature.

In the present study the phytochemical analysis of *Crinum defixum* Ker-Gawler leaves has revealed a variety of chemical constituents such as alkaloids, flavonoids, steroids, acids, fatty acid esters and phenolic compounds are produced by this plant, the major phytoconstituent being amaryllidaceae type of alkaloids. In the current investigation the four new alkaloids of Piperazine, 1,4-bis (2-methoxybenzoyl)- (Ethanol fraction), Piperazine-1-carboxylic acid, 4-(2-fluoro-4-(1-oxopropyl) phenyl)-, ethyl ester (Methanol fraction), Ethanone,1-(2,5 diphenyl-2H-1,2,3-triazol-4-yl 3-oxide)-, oxime and 7-(5-Cyclohexylpentyl)-6-hydroxy-5,8-quinolinedione (Acetone fraction) and the flavonoids of 4',5,7-Trihydroxy isoflavone i.e., Genistein (Chloroform, ethyl acetate, butanol and methanol fractions) and 4H-1-Benzopyran-4-one, 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy i.e., Quercetin

(Ethyl acetate fraction) are first time identified from leaves of *Crinum defixum* Ker-Gawler under present study. The other non-alkaloidal constituents of steroids, fatty acid esters, acids and phenolic compounds are also identified the same. The identified compounds were used to determine their chemical and biological properties using Dr. Duke's phytochemical and Ethnobotanical databases, NCBI-Pubmed, Chem Spider (Royal Society of Chemistry) and other available literatures.

The identified compounds are which contribute the activities like Antioxidant, Antimicrobial, Cancer preventive, Anemiagenic, Antiandrogenic, Therapeutic, Diagnostic, Antitumor agent, antiangiogenic and immunosuppressive, Analgesic and anti-inflammatory, Hypercholesterolemic, Nematicide, Antibacterial, Insectifuge, Dermatitigenic, Antifungal, Pesticide, Lubricant, Flavor, Hemolytic, 5-Alpha reductase inhibitor and Androgenic alopecia (hairloss) activities. Hence the plant *Crinum defixum* Ker-Gawler has a potential source of biologically important drug candidates.

## 7. Conclusion

The preliminary phytochemical analysis of various extract of *Crinum defixum* Ker-Gawler leaves contains many bioactive chemicals like alkaloids, flavonoids, saponins, terpenoids, amino acids and phenolic compounds (table-1). The *Crinum defixum* Ker-Gawler has rich in (Amaryllidaceae) alkaloids content in both bulb and leaves. The Amaryllidaceae



alkaloids exhibit a range of biological activity, both pharmacological and microbiological. Among the most noted effects are: analgesic, central nervous system, anti-tumor, antiviral and anticholinergic. Hence the medicinal plant *Crinum defixum* Ker-Gawler leaves has a Phytochemically potent one.

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