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GCMSD ALS ANALYSIS OF BIOACTIVE COMPOUNDS IN FERMENTED SAUERKRAUT

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ABSTRACT

Background: The presence of diverse secondary metabolites (phytochemicals) has been reported from plants of the genus *Brassica*. The researchers found that the process of lactic acid fermentation of sauerkraut with raw cabbage (*Brassica oleracea (*var.*capitata)*) produces glucosinolates (GLS) and isothiocyanates, two major groups of phytochemicals. These compounds that stimulate phase 2 detoxication enzymes improve antioxidant status and protect animals against chemically induced cancers. Natural occurrences of some phytochemicals in sauerkraut also described to exhibit antidiarrheal, bactericidal, fungistatic, fungicidal and pesticidal actions to plants and animals. Objective: This study was designed to determine the bioactive phytochemicals from crude extracts of sauerkraut by using GCMSD ALS (Gas Chromatographic Mass Spectrometry Detector Automatic Liquid Sampler) method. **Materials and Methods:** GC-MS analysis of the whole fermented sauerkraut was done using gas chromatography-mass spectrometry -Acquisition SW Version Mass Hunter GC/MS Acquisition B.07.06.2704 analyzer. **Results:** This investigation was carried out to determine the possible bioactive chemical compounds from fermented sauerkraut by GCMSD ALS method. Twenty three different volatile aldehydes, ketones, furans, acids, alcohols, esters, branched chain amino acid derivatives, phenylalanine, sulphides, pyrazines and other compounds were discovered in the crude extract samples of sauerkraut. This analysis revealed that the crude extract of sauerkraut contain some bio active compounds like ; S-Methyl methanethiosulfonate, Propionic acid, 2-oxo-, methyl ester, Acetic acid, 4H-Pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl, 5-ethenyl-2-methoxypyrazine, Benzene acetaldehyde , Disulfide dimethyl, Dimethyl trisulfide etc. The antimicrobial, anti-inflammatory, antiviral and antiproliferative properties of these derivatives have been reported in different studies. Propionic acid is used as a common preservative or stabilizer to avoid decomposition by microbial growth or by undesirable chemical changes in many of the animal and human foods. **Conclusions:** From the GC MS spectrum of sauerkraut sample, it is marked that most of the bioactive chemical compounds identified by GCMSD ALS method are documented as active agents in chemotherapy of different types of cancers. It is demonstrated that the sauerkraut – the fermentation probiotic product prepared from *B. oleracea* var. *capitata* contains different bioactive secondary metabolites like glucosinolates , isothiocyanates and flavonoids and possessed diverse antioxidant, antibacterial, antifungal, pesticidal and anticancer properties.

KEYWORDS: Glucosinolates, isothiocyanates, bioactive, GCMSD ALS, sauerkraut, *B. oleracea* var. *capitata*.

INTRODUCTION

Glucosinolates are sulfur- and/or nitrogenous secondary metabolites, primarily present in the plants of *Brassicaceae* family (Kushad M 1999). Raw cabbage is normally rich in glucosinolates and isothiocyanates - both been objects of research for more than half a century. The researchers found that during the fermentation process enzymes are released that completely decomposes these glucosinolate bioactive compounds into several breakdown products. Antimicrobial, antioxidant and anti-inflammatory activities of isothiocyanates and other sulphur compounds originating from *Brassica* vegetables have also been reported (Kyung & Fleming, 1997; Lin *et al*., 2008; Mastelic *et al* 2010). Free radicals or reactive oxygen species (ROS) generated from various sources in the environment as well as from cellular processes in the body are of serious health challenges (Engwa G 2018). Phytochemical compounds with antioxidant properties have the ability to inhibit the damages caused by reactive oxygen species. The antioxidant properties of sauerkraut depend on the presence of various bioactive compounds produced by the metabolic action of probiotic lactic acid bacteria during fermentation. Fresh raw cabbage and its

fermentation product sauerkraut is rich source of phenolic content, vitamins, carotenoids, flavonoids and other phytocomponent constituents. Because of this positive aspect experimental study was focused on exploit the potential of phytochemicals constituents from sauerkraut extracts and results were coordinated for their involvement with health benefits.

MATERIALS AND METHODS

Preliminary Sample Collection & Preparation

A total 10 samples of cabbage heads (*Brassica oleracea var.capitata)* around 500 gram of average weight were collected randomly from supermarkets / town markets of Pune district. Sample collection was done according to the instructions given in The Food Safety and Standards Authority of India (FSSAI) Ministry of Health & Family Welfare, Government of India. The fresh cabbage samples were packed into sterile plastic containers, transported to research center and further experiments were carried out immediately to prevent deterioration. Outer leaves of cabbage heads were discarded and each cabbage head was rinsed in mild soap under running cold water and allowed to drain. Cabbage head was cut quarterly leaving the core in and finely sliced into small thin even pieces (length of 1.5 cm and 2 – 2.2 mm thickness) with the help of food grinder in sterilized area. 5 kg of shredded cabbage was collected in large sterile container for mixing. Total 100 grams (2%) of canning non ionized salt was sprinkled over the chopped cabbage slices. This salted cabbage was allowed to stand 5-10 minutes to wilt slightly. Salt helps to draw liquid juices out from the cabbage. Then the whole mixture was thoroughly mixed and transported to specially designed sterilized fermentation container and was pressed properly so that the shredded cabbage covered with 1-2 inches of its own juice (brine). Lactic acid fermentation of sauerkraut was carried out by natural micro flora present on the cabbage leaves. The lid of the fermenter vessel was covered with clean dry sterile cloth and fermentation process was carried out at room temperature 27- 30° C for the next thirty days in clean dry place. During fermentation process number and types of various lactic acid bacteria and pH of the fermentation medium were recorded for confirmation of ideal process.

Preparation of Extracts

Method described by Amarowicz *et al* (1995a) was monitored with slight modifications for preparation of sauerkraut extracts. Lyophilized sauerkraut samples were grinded in a blender (Hamilton Beach Hbh550 Series Fury Electric Blender) to fine particles along with small quantity of pure water. Crude aqueous extract was vigorously shaken for 5 mins and was kept at 4^0C for 6 hrs. This crude aqueous extract was aseptically transferred to heat resistant stoppered containers (50ml capacity) with the solvent (1: 10 v/v 50% methanol). It was kept for a period of 3 hrs at room temperature. Equal amount of boiling water was added and stoppered containers were heated at 60°C temperature for 15 mins. The mixture was then filtered through double set of 0.22 micro membrane filters (Whatman™ 1001-090 Grade1Whatman/GE Healthcare). In next step filtrate was concentrated at 50°C and subjected to column chromatography. Eluents fractions were separated by using silica gel column with increasing concentrations of isopropyl alcohol. After initial fractionation, TLC (thin layer chromatography) technique was monitored for each fraction to distinguish individual compounds in each separated fraction (Silverman *et al* 2014; Mortensen *et al* 2015). Phytochemicals appropriate fractions (monitored by TLC) were collected in separate stoppered containers for further study.

Preliminary Bioactive compounds Screening

The methanol extract of sauerkraut was tested for the identification of diverse volatile secondary metabolites like glucosinolates, isothiocyanates, phenolic, flavonoids and glucosinolate breakdown products by GCMSD ALS method.

GCMSD ALS Analysis of Sauerkraut

The bioactive compounds investigation of methanolic extract of sauerkraut was performed on GCMSD ALS equipment. Acquisition SW Version Mass Hunter GC/MS Acquisition B.07.06.2704 1989-2017 Agilent Technologies, Inc. Experimental conditions of GC-MS system were as follows: Expected Barcode Sample Amount - Dual Inj Vol 0.5; Tune Name - etune.u Tune Path - D:\MassHunter\GCMS\1\5977\; Tune Date - Stamp 2019-03-26T09:31:33+05:30 MS Firmware Version - 6.00.34;

RESULTS AND DISCUSSION

The results pertaining to GC-MS analysis of the methanolic extract of sauerkraut made up from *Brassica oleracea var.capitata* lead to the identification of a number of biologically active compounds. These compounds were identified through SW Version Mass Hunter gas chromatography mass spectrometry (GC/MS). The various compounds present in the crude extract sample of sauerkraut that were detected by the GC-MS are shown in table 1 along with DB formula and (Lib) score. Borane-methyl sulfide complex, Butanal, 3-methyl, Butanal, 2-methyl, Disulfide, dimethyl, Acetic acid, Propanoic acid, 2-oxo-, methyl ester, 2,3-Butanediol, 2,3-Butanediol, [R-(R*,R*)], Methylamine, N,N-dimethyl, Guanidine carbonate, Dimethyl trisulfide, 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, S-Methyl methanethiosulfinate, 2H-Pyran-2,6(3H)-dione, 1H-Pyrrole-2-acetonitrile, 1-methyl, Benzene acetaldehyde, 3(2H)-Furanone, 4-hydroxy-5- methyl, S-Methyl methane thiosulphonate, Furaneol, 4H-Pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl,4H-Pyran-4-one,3,5-dihydroxy-2-methyl,5(Hydroxy-methyl)dihydro-furan-2(3H)-one;5-ethenyl-2-methoxypyrazine, were present in the methanolic extracts of fermented sauerkraut. The composition determined for this methanolic extract corresponds to 79.26% of the entire GC-MS chromatogram.

The GC-MS spectrum + EI TIC Scan confirmed the presence of various bioactive compounds with different retention times (Counts vs. Acquisition Time min) as illustrated in fig.1 The GCMSD ALS analyzes the compounds eluted at different times to identify the nature and structure of the individual compound. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are compound chromatograms or fingerprint of that compound which can be identified from the data library NIST17.L. Isothiocyanates and their glucosinolate precursors are widely distributed in higher plants and are especially prevalent among cruciferous vegetables (Fenwick G *et al* 1983). Certain natural and synthetic aromatic isothiocyanates have been known for more than a decade to inhibit mammary, fore stomach, and lung tumorigenesis induced by polycyclic aromatic hydrocarbons in rodents (Wallenberg I 1985). Thiosulfinates which are found in most of the *Alliums* and other vegetable plants act as unstable intermediates in the enzymatically initiated degradation of S-alk(en)yl-L- cysteine sulfoxide. At the pole position of this discussion, we discovered reactive sulfur species such as thiosulfinates, disulfides, polysulfanes, and isothiocyanates. Some of these sulfur-based agents are pharmaceutically important and already used in chemotherapy. Dietary administration of S-Methyl methanethiosulfonate (MMTS), isolated from cauliflower, during the post initiation phase inhibited the incidences of intestinal neoplasms induced by AOM (azoxymethane) in rats. Also, MMTS reduced the formation and the growth of colonic ACF (Aberrant crypt foci) and inhibited expression of several cell proliferation biomarkers like Brd Urd-labeling index, and blood polyamine levels in the long-term experiments. These results suggest that MMTS might be a possible chemo preventive agent for colon cancer (Kawamori T 1995) and inhibited the development of both preneoplastic and neoplastic colonic lesions induced by AOM. The suppressive effect of MMTS on aflatoxin B1 (AFB1) - or methyl methanesulfonate (MMS)-induced chromosome aberrations (CA) in rat bone marrow cells was studied by Ito Y *et al* in 1997. MMTS significantly suppressed CA induced by both AFB1 (an indirect-acting carcinogen) and MMS (a direct-acting carcinogen) (Ito Y1997). Vladimir *et al* in 2019 reported examples of MMTS application in experiments involving oxidoreductase (glyceraldehyde-3-phosphate dehydrogenase, GAPDH), redox-regulated protein (recoverin) and cysteine protease (triticain-α) (Vladimir 2019). Pyrazolone derivatives such as antipyrine, aminopyrine, and dipyrone are well known compounds used mainly as analgesic and antipyretic drugs and their pharmacological molecular mechanism has been widely surveyed (Himly, M 2003 ; Gursoy, A 2000). One of the best known antipyrine derivatives is 4-aminoantipyrine which is used for the protection against oxidative stress as well as prophylactic of some diseases including cancer, and these are important directions in medical applications (Teng, Y2011). Several derivatives of antipyrine were also biologically evaluated, and analgesic (Turan-Zitouni, G 2001), anti-inflammatory (Lutsevich, A 1995), antimicrobial (Bondock, S.2008), and anticancer activity (Metwally, M. 2012; Kakiuchi, Y. 2004; Sigroha, S 2012) have been reported. Antipyrine derivatives are strong inhibitors of cycloxygenase isoenzymes, platelet tromboxane synthesis, and prostanoids synthesis (Chandrasekharan, N. 2002), which catalyze the rate-limiting step of prostaglandin synthesis. Pyrazolones are also a well-known elicitor of hypersensitivity (Levy, M 2000). Some of the studies reported that 4H-pyran-4-one, 2, 3-dihydro-3, 5-di hydroxy-6-methyl and 2H-pyran-2-one, 4,6-dimethyl are flavonoids pyrones which has been isolated from the aqueous extract of *Vitex negundo* , *Cyperus rotundus* and *Helichrysum italicum* leaves have shown antimicrobial, anti-inflammatory, antiviral and antiproliferative properties. From this study following active flavonoids pyrones were identified from sauerkraut ethanol extract - 2H-Pyran-2,6(3H)-dione , 1H-Pyrrole-2-acetonitrile, 1-methyl , 4H-Pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl, 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl, 5-ethenyl-2-methoxypyrazine. Propionic acid (PA) is a fungicide and bactericide, registered to control fungi and bacteria in stored grains, hay, grain storage areas, poultry litter, and drinking water for livestock and poultry. European Union (EU) certifies PA as the great of grain preserver and most efficient in controlling *Salmonella* and other pathogens (Haque, M 2009). Acetic acid & Propionic acid, 2-oxo-, methyl ester identified in this study at retention time 3.033 & 4.107 respectively. Benzene acetaldehyde is an aromatic chemical compound with antioxidant and anti-inflammatory activities (Kochi M 1980). Benzene acetaldehyde, Disulfide, dimethyl, Methylamine, N, N-dimethyl & Dimethyl trisulfide are responsible for the antibiotic activity of maggot therapy, which also imparts floral fragrances during treatment. These compounds are used as antimicrobial agents in plant pathology which inactivates fungi, bacteria and *M. incognita* -cotton root-knot nematodes. In 2019 Lihua Tang *et al* demonstrated first study in which suppression of the infection process was carried out by Dimethyl trisulfide against *C. gloeosporioides* on mango fruit & thus providing a dimethyl trisulfide as a novel post-harvest bio rational control for mango anthracnose a fungal infection. (Tang L 2019) In current study benzene acetaldehyde & dimethyl trisulfide was identified at retention time of 7.807 & 6.579 respectively by GCMSD ALS analysis. Molecular biology experimental results demonstrate that interactions of compounds present in sauerkraut extracts with the SH_2 domain of $STAT_3$ might be accountable for their inhibitory effects.

Boranedimethylsulfide (BMS) is a complex of borane with dimethylsulfide. The other common complex of borane is tetra hydrofuraneborane complex. Boranedimethylsulfide complex is more stable than tetra hydrofuraneborane complex and is therefore available in higher concentrations in fermented sauerkraut extract.

Borane dimethylsulfide complex acts as a reducing reagent in different types of biochemical reactions. Experimental studies revealed that dimethyl disulfide (DMDS), a plant-derived insecticide, is a promising soil fumigant in farms and crops as a substitute for synthetic gas fumigant agent like methyl bromide. These compounds affect multiple targets, which could be an effective way to improve pest control efficacy of fumigation. In journal of molecular cell biochemistry (2006) Arumugam Arunkumar and his colleague has published a research which defines the role of diallyl disulfide (DADS) obtained from garlic compounds to induce cell cycle arrest in prostate cancer cell line PC-3. In this study, diallyl disulfide (DADS) was studied for its antiproliferative and induction of cell cycle arrest on prostate cancer cells *in vitro*. Most of the phytochemicals identified in this study by GC-MS method are recorded as active agents in chemotherapy of different types of cancers. It is demonstrated that the sauerkraut – the fermentation probiotic product prepared from *B. oleracea* var. *capitata* contain different phytochemical compounds and possessed diverse antioxidant, antibacterial, antifungal and anticancer properties. Detailed studies on effect of fermentation products on different cell lines and further clinical trials will be the future plan of this research. The current study helps to predict the DB formula and constitution of total 23 bioactive compounds. Further research may guide to seclusion of individual bio-active compounds and their structural illumination. Detail study of screening of pharmacological actions of these bioactive compounds will be helpful for further drug development against pathological species of bacteria, fungi and nematodes.

CONCLUSION

The presence of various bio-active compounds detected after GC-MS analysis using the methanolic extract of sauerkraut justifies the use of this fermented probiotic product by traditional practitioners. However, separation of discrete phytochemical elements and imperiling it to the biological action will be certainly giving productive results and will built a new area of research of individual constituents and their pharmacological influence. The present study suggests that anticancer effect of fermented sauerkraut would be attributed to the various bio- active compounds, - S-methyl methanethiosulfonate, Methyl methanethiosulphonate, Dimethyl trisulfide and Propionic acid, 2-oxo-, methyl ester derivative. Assessment of pharmacological action is under development. Fermented sauerkraut can be recommended as a probiotic food of phytopharmaceutical importance.

Table 1: Identified Compounds with DB Formula, Molecular weight & Retention time.

Sr. No.	Compound Label	DB Formula	Molecular Weight	Retention Time
1	Borane-methyl sulfide complex	C_2H_9BS	75.96	1.28
2	Butanal, 3-methyl	$C_5H_{10}O$	86.13	1.981
3	Butanal, 2-methyl	$C_5H_{10}O$	86.13	2.065
4	Dimethyl disulfide	$C_2H_6S_2$	94.19	2.951
5	Acetic acid	$C_2H_4O_2$	60.05	3.033
6	Propanoic acid, 2-oxo-, methyl ester	$C_4H_6O_3$	102.08	4.107
7	2,3-Butanediol	$C_4H_{10}O_2$	90.12	4.248
8	2,3-Butanediol, [R-(R*,R*)]	$C_4H_{10}O_2$	90.12	4.342
9	N,N-dimethylmethanamine	C_3H_9N	145.25	5.017
10	Guanidine carbonate	$C_3H_{12}N_6O_3$	180.18	5.533
11	Dimethyl trisulfide	$C_2H_6S_3$	62.13	6.579
12	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144.12	6.931
13	S-Methyl methanethiosulfinate	$C_2H_6OS_2$	110.18	7.047
14	2H-Pyran-2,6(3H)-dione	$C_5H_4O_3$	224.64	7.2
15	1H-Pyrrole-2-acetonitrile, 1-methyl	$C_7H_8N_2$	120.15	7.394
16	Benzeneacetaldehyde	C_8H_8O	120.14	7.807
17	3(2H)-Furanone, 4-hydroxy-5- methyl	$C_5H_6O_3$	128.12	8.063
18	Methyl methanethiosulphonate	$C_2H_6O_2S_2$	126.18	8.258
19	Furaneol	$C_6H_8O_3$	128.12	8.324
20	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	144.12	9.574
21	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl	$C_6H_6O_4$	142.10	10.178
22	5-(Hydroxymethyl)dihydrofuran-2(3H)-one;	$C_5H_8O_3$	116.11	10.277
23	5-ethenyl-2-methoxypyrazine	$C_7H_8N_2O$	138.16	11.062

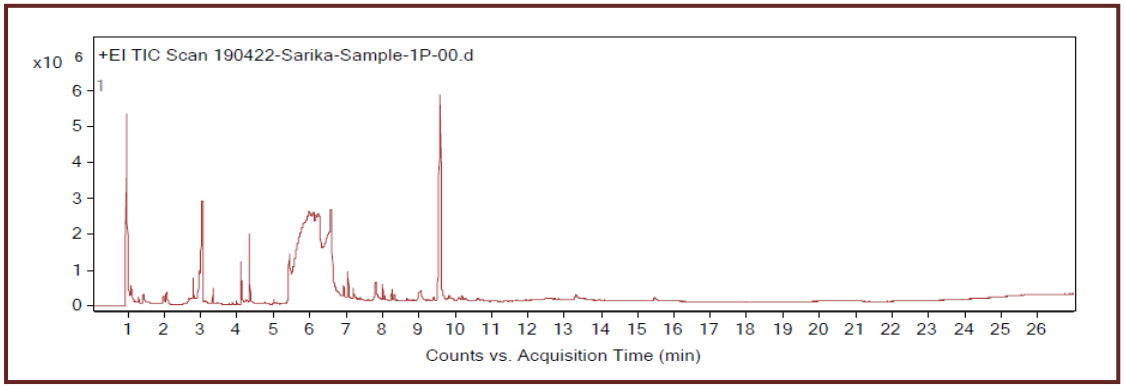
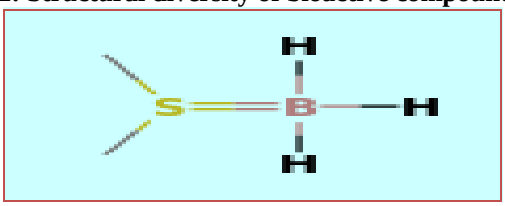
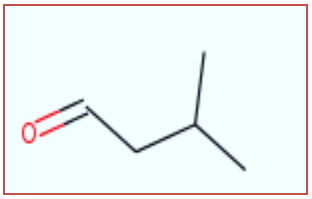


Fig. 1: GC MS Spectrum of Sauerkraut Sample.

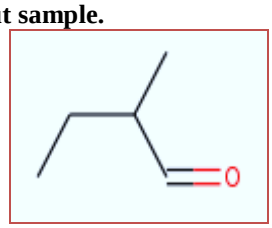
Fig. 2: Structural diversity of bioactive compounds in GCMS spectrum of sauerkraut sample.



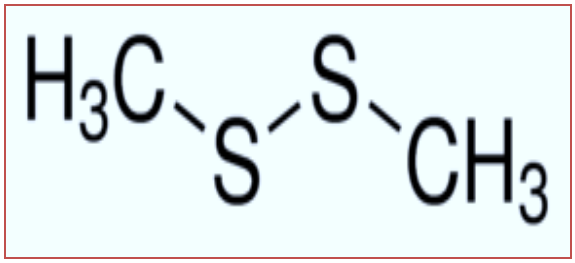
Borane-methyl sulfide complex (1)



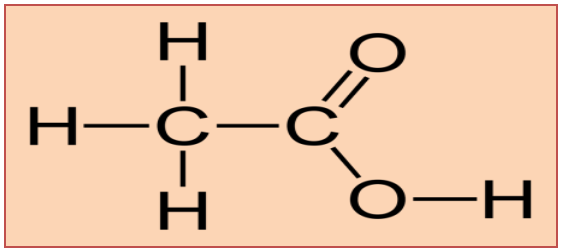
Butanal, 3-methyl (2)



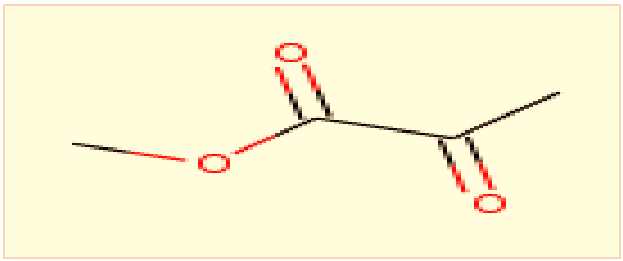
Butanal, 2-methyl (3)



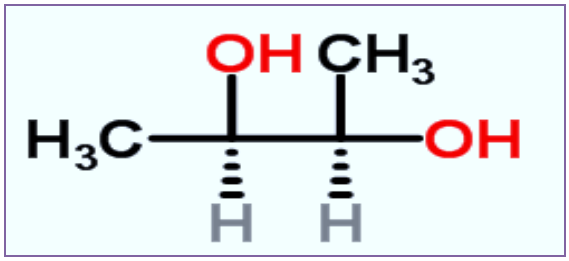
Dimethyl disulfide (4)



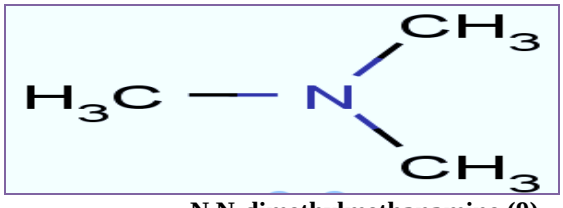
Acetic acid (5)



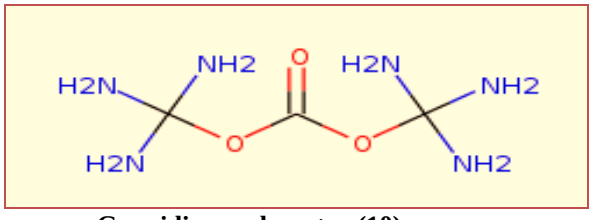
Propanoic acid, 2-oxo-, methyl ester (6)



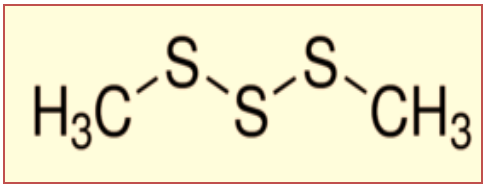
2, 3-Butanediol, [R-(R*, R*)] (7,8)



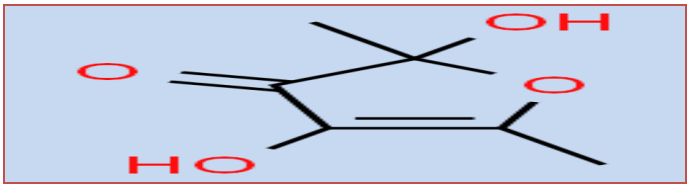
N,N-dimethylmethanamine (9)



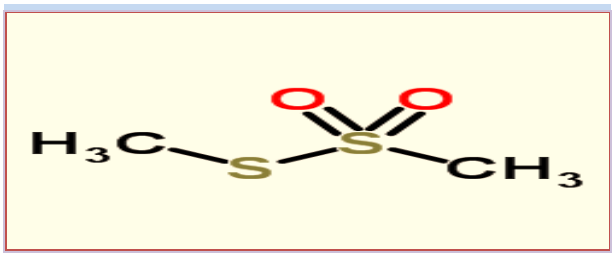
Guanidine carbonate (10)



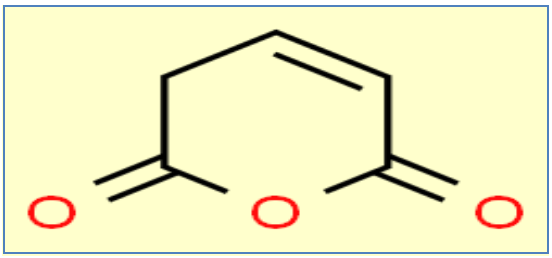
Dimethyl trisulfide (11)



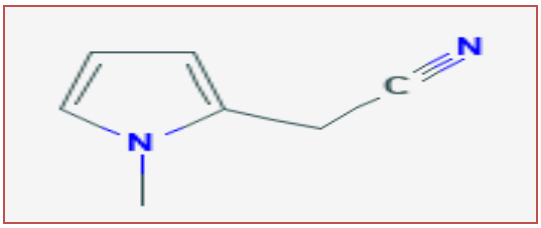
2, 4-Dihydroxy-2, 5-dimethyl-3(2H)-furan-3-one (12)



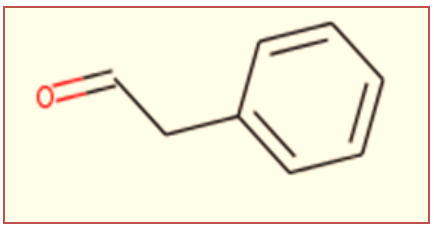
S-Methyl methanethiosulfonate (13,18)



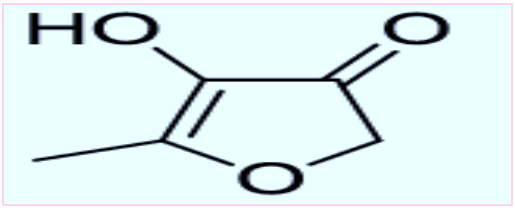
2H-Pyran-2, 6(3H)-dione (14)



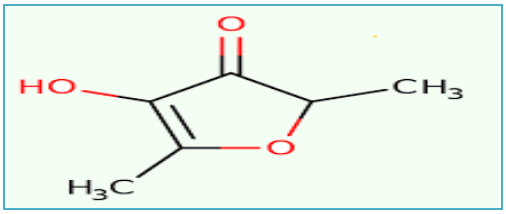
1H-Pyrrole-2-acetonitrile, 1-methyl (15)



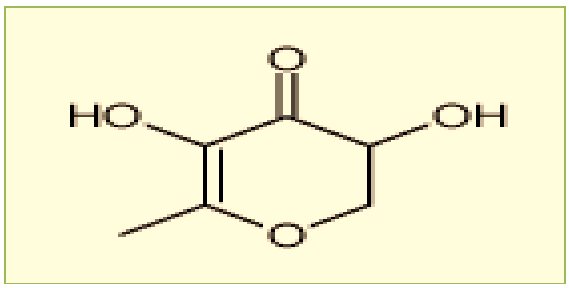
Benzeneacetaldehyde (16)



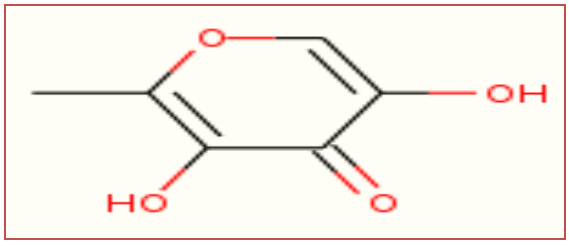
3(2H)-Furanone, 4-hydroxy-5- methyl (17)



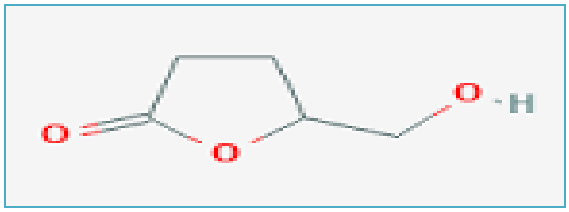
Furaneol (19)



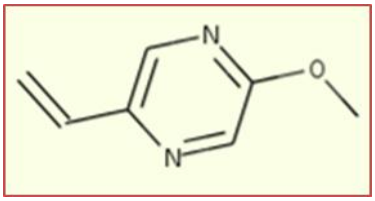
4H-pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl (20)



4H-Pyran-4-one, 3,5-dihydroxy-2-methyl (21)



5-(Hydroxymethyl) dihydrofuran-2(3H)-one (22)



5-ethenyl-2-methoxypyrazine (23)

Footnotes

Declarations

Availability of data and materials

The data that support the findings of this study are available from the corresponding author on request. Requests for the data from this study can be submitted via email to sarikamohol@gmail.com.

Competing interests

The authors declare no conflicts of interest.

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Authors' contributions

Conceptualization, Methodology, Investigation, Writing—original draft, by Sarika Bhosale Corresponding author.Writing—review and editing and supervision by Dr. Vaijayanti Sapre .The authors read and approved the final manuscript.

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Consent for publication

Not applicable.

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