ESTIMATION OF VOLATILE CONSTITUENTS IN ROSE WATER BY HS-GC-MS AND STUDIES ON SAFETY PROFILE AND CYTOTOXICITY

[1]Safia Abidi, [2]Sobia Salman, [3]Faizah Mughal, [4]Suleha Jabeen, [5]Sumreen Mujahid, [6]Zafar Alam Mahmood, [7]Salman Tariq khan, [8]Abdul Hafeez Laghari, [9]AishaNelofar, [10]Syed Waleed Ahmed Bokhari

[1]Safia Abidi
Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan.

[2]Sobia Salman
Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences. University of Karachi, Karachi, Pakistan.

[3]Faizah Mughal
Department of Biochemistry, Fazaia Ruth Pfau Medical College (FRPMC), PAF Base Faisal, Karachi, Pakistan.

[4]Suleha Jabeen
Department of Pharmacognosy Faculty of Pharmacy and Pharmaceutical Sciences. University of Karachi, Karachi, Pakistan.

[5]Sumreen Mujahid
Department of Pharmacology , Baqai Medical College, Baqai University, Karachi, Pakistan.

[6]Zafar Alam Mahmood
Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences. University of Karachi, Karachi, Pakistan.

[7]Salman Tariq khan
PCSIR Laboratories Complex Karachi, Pakistan.

[8]Abdul Hafeez Laghari
PCSIR Laboratories Complex Karachi, Pakistan.

[9]AishaNelofar
PCSIR Laboratories Complex Karachi, Pakistan.

[10]Syed Waleed Ahmed Bokhari
Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences. Hamdard University, Karachi, Pakistan.

Author's Contribution:
S.A., S.S and Z.A.M. architect the design. S.J. did computational framework and analyzed the data. F.M. and A.H.L. carried out the implementation. S.T.K. and S.M., performed the calculations. A.H.L. also wrote the manuscript with input from all authors. A.N. and S.W.B. conceived the study and were in charge of overall direction and planning.

Corresponding Author:
Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan.

ABSTRACT:

Background:

Rosa damascene mill is the most commonly used plant because of diverse secondary metabolites. Therefore it was taken in to consideration to determine the vital components of the aqueous extract of flower petals to carry out acute toxicity studies of rose water.

Material and method:

The volatile components were studies through HS-GC-MS (headspace-gas chromatography-mass spectrometry).

Results:

The presence of total forty-six constituents in sample-1, thirty-eight constituents from sample-2 and sample-3. Among all constituents, ten were common and highlighted as phenyl ethyl alcohol, citronellol, gereniol,nerol, pentadecane, heptadecanol, octadecanol, tetracosane, decane and nonane. The most important compounds identified were citronellol, gereniol, nerol and phenyl ethyl alcohol. Results of the acute toxicity studies showed that all animals subjected at 15 ml/70kg, 20 ml/70kg and 25ml/70kg adult dose indicated no signs of any irregularities in any test animals during study duration.The brine shrimp lethality bioassay result reflected that all rose water samples tested had LC_{50} >3000µg/ml showing practically non-toxic.

Conclusion:

From the results, it is evident that *rose water from Rosa damascene Mill* containing various volatile compounds has strong safety profile, justifying its uses and applications in various phytopharmaceutical formulations.

Keywords: Rose Flower, HS-GC-MS, Fragrance, Volatile Compounds sample, Peak Area, Acute toxicity study, Brine Shrimp Lethality Test.

INTRODUCTION:

Rosa damascene had a long history of use in the traditional system of medicine as well as in cosmetic industries due to its fragrance property (*Abidi et al*., *2018*). This specie of rose was also known as oil-bearing rose, used mainly for the production of rose water and rose oil by the method of steam distillation or as solvent extraction which produces rose concrete and rose absolute (*Badzhelova et al*.,*2017*). Morethan 400, biochemical volatile compounds have been so remote recognized and report from rose. The chemical compounds of the rose have been classified in to five classes' carbohydrates, alcohol, esters hydrocarbons, aromatic esters and others. Each compound have been reported to passes different chemical properties thus have and application (*Atanasova et al., 2016*). The reimbursement of rose water are various; it take action asan pain reliever (*Boskabady et al.,2011*) , relaxing agent along with anti-inflammatory , Rose water help in shielding skin on or after the injurious effects of UV emission . Rose water is affluent resource of anti-oxidants which helps in the building of the membrane tissues and offer supremacy to membrane cell also incorporated in perfume, collyrium and lotion, vocation as a blood purify representative used in numerous formulation to care for skin complaint, bad skin and improves skin complexion (*Rizvi et al., 2016*). It acts as a gentle soothing, frame of mind attractive manager and relieve nervousness, rose water has antitussive effect ,used as a hair stimulant, bug repellant.Rose water is extensively used to manage and deal with eye infections. additional special types of beauty formulations (soaps, lotions, cream cleanser) utilisis rose water , it is also integrate in ointment as emollient turn out cooling effect(*Abidi et al*.,2018) . The chief constituent is phenyl ethyl alcohol, citronellol, gereniol, nerol , pentadecane, heptadecane,octadecanol, tetracosane, decane and nonane give outstanding and satisfying aroma and pharmacological action to the rose water.The measure of phenyl ethyl alcohol differ in rose water , extract from rose petal by hydro distillation method (*Mahboubi et al.,2016*).

Globally, rose water has shown a wide range of usage and application proven efficacy and thus demands the researchers to investigate and explore the safety profile for its secure custom and application(*Agarwal et al .,2005*). Keeping in view of all these factors objectives of the current learning was planned to calculate approximately the volatile constituents of rosewater samples and to establish its safety profile using short term toxicity examination.

Acute toxicity cause by a moderator as soon as administer in one, or further dose extra than a stage not greater than 24 hours, involve detrimental assets to the living being all the way through single or little period contact. Study worn during the range of preliminary doses for phase-I individual and creature studies, offer information associated to acute overdose in humans and animals. The test stand on the path of material administers to the animal and therefore it is classified from Class-1 to Class-5 for oral, dermal, gas inhalation, vapour/dust/mist inhalation and injection. dose repetitive throughout the administration of investigation substance via variety of routes of experience, including gavaging which involve abdomen intubation or enforced feeding, inoculation, membrane, painting, and gulp of air (*OECD Guideline for Testing of Chemicals. 2001*). Toxicity testing of the herbal extract was same as the conventional medicine, both *in-vitro* and *in-vivo* methods employed for the toxicity testing, *in-vivo* methods are done on mice and rats whereas *in-vitro* toxicity test perform by by means of a model such as BST (Brine shrimp lethality test). The main advantage of this toxicity testing is the use of shrimp, there is much homogeneity in the eggs, nauplii are extremely responsive to chemicals (*Apu et al.,2010*).

MATERIALS AND METHODS:

Rosewater sample

Rosewater sample#1,was provided by Mohammad Hashim Tajir Surma Karachi Pakistan.Sample#2 was an unbranded product purchased from local market, while Rosewater sample# 3 was prepared in the lab.

Recognition of Rose Flower

Rose flower sample purchase from the general market (Karachi), authenticated by Professor Dr. Muhammad Mohtasheemul Hasan, Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, Karachi, Pakistan, as Damask rose*(Rosa damascena Mill.),* family Rosaceae. voucher of the sample (RD-01-12)obtainable from "Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi herbarium".

Preparation of RoseWater

In the lab, rosewater ready as lot production. Ten kilogram fresh rose petals be procure from general marketplace .after clean-up, and cataloging contaminations shade dried up at room temperature (25^{o}C) till the dampness approximately 79.30% was detached. Petals spreaded over nylon sieves. The period of drying procedure last for twelve days. The dried petals was kept back in amber coloured airtight glass jars. Dried rose-petal (60g) was added into 1.5L of purify water and distilled for four hours. about 800ml(on average basis) of rose water, recovered during each distillation process (*Abidi et al.,2018*).

Determination of volatile constituents

Determination of volatile constituent, rosewater was extracted with petroleum-ether, 2 steps involved. 1- Step 500ml of rosewater was mixed with five hundred milliliter of petroleum- ether and the mixture heated to forty five degree Celsius for 150 minutes using a water bath to shift the volatile constituents from aqueous to organic phase. 2- Step, the residue of the organic phase from first step removed,further five hundred milliliter of fresh petroleum- ether added into the system. The content was again heated to forty five degree Celsius for 150 minutes to complete the extraction procedure. The volatile components extracted from this procedure, further concentrated in a rotary evaporator equipped with a vacuum pump (*Moein et al.,2010*).

Instrumentation:

HS-GC-MS Analysis (Headspace-Gas Chromatography-Mass Spectrometry)

Headspace coupled with GC-MS (Shimadzu 2010, Singapore 118227) used for sample agitation; incubation time was twenty minutes at one hundred and twenty degree Celsius. Partition of constituent achieve with a DB-5 MS capillary column (30 m, 0.25 mm i.d., 0.25 @m) coupled directly to mass detector;MS spectra recorded by electron impact mode (70 eV). The sample injected into the splitless mode at two hundred and twenty degree Celsius. The initial column temperature was fifty degree Celsius, maintained for five minutes then programmed to increase at five degree Celsius to two hundred and forty degree Celsius. Data acquisition performed in the full-scan mode (m/z range 35-600) after optimisation of the MS.

Identification of Phytoconstituents

Interpretation on mass-spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST).

Acute toxicity studies

Experimental Animals

Albino rats (wistar),187-196 g in weight obtained from " Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences,University of Karachi".Animals acclimatised to regular laboratory circumstances that are twelve hours light and twelve hours dark cycle, feed standard diet, Animals was fasted twelve hours prior to dosing. The institutional animal ethical guiding principle was strictly followed.

Experimental Design

Experiment conducted over the principle of Organization for Economic Co-operation and Development 423. (*OECD 423, Paragraph 23*).Total of 12 animals divided into 4 dosage groups with 3 animals per dose. Group-1 serves as control (normal saline), group-2 received dose of 15ml/70kg, Group-3 received dose of 20ml/70kg and Group-4 received dose of 25ml/70kg. Doses planned according to the body weight of rat. Dosing was performed by means of the ball –tipped intubation needle affixed with 1 ml syringe, observed for behavioural alteration and other toxicity symptoms. Results were observed for thirty minutes followed by an hourly interval of up to forty eight hours (*Amna et al.,2013*).

Brine shrimp (Artemia Salina) lethality

Brine shrimp lethality assays performed at ICCBS(the International Center for Chemical and Biological Sciences), University of Karachi, by following the method as described by (*Olowa et al.2013*),.Different concentration of rosewater samples arranged by obtaining the final concentration of 10, 100, 1000 µg/ml. 10 napuliis with the help of the pipette collected from the lighter side of the hatching compartment, put into vials containing the different concentrations of rosewater extract and 4.5 ml of seawater. Standard drug etoposide was used. The control setup contained 4.5ml of seawater 10 napuliis and 0.2percent of distilled water. After 24 hours each vial was observed with the help of magnifying glass and counts the number of survivals and data recorded.

STATISTICAL ANALYSIS:

Test results, communicated as mean standard deviation, SPSS adaptation 20.0, apply

RESULTS:

HS-GC-MS Analysis Of Volatile Constituents

The volatile compounds of rose water samples listed in table 1, 2 and 3 while graphically represented in Figure.1,2 and 3. In headspace analysis compound were identified as monoterpene, aldehyde and alcohol while phenyl ethyl alcohol, citronellol,geraniol and nerol identified as a significant compound. The other significant compounds were pentadecane, heptadecanol, octadecanol, tetracosane, decane and nonane. The rose water has the characteristic and distinct odor due to the presence of the phenyl ethyl alcohol.

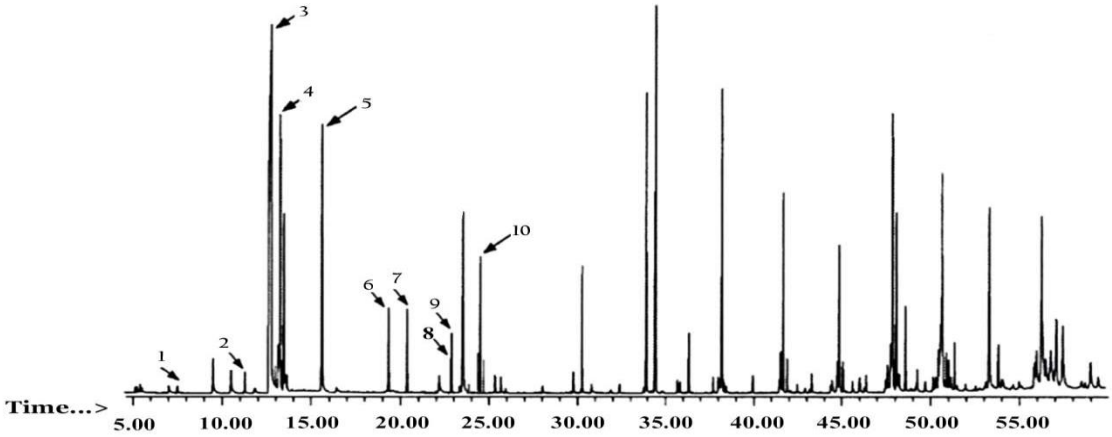

FIG.1: GC-MS ANALYSIS OF SAMPLE-1

TABLE 1: COMPOUND PRESENT IN GC-MS ANALYSIS OF SAMPLE-1

S.NO	COMPOUND	RETENTION TIME	% of V.C (*)
1.	NONANE	7.5	0.2
2.	DECANE	11.20	0.34
3.	PHENYLETHYLALCOHOL	12.594	5.08
4.	PENTADECANE	13.367	8.82
5.	CITRONELLOL	15.867	3.06
6.	NEROL	19.600	1.5
7.	GERENIOL	20.160	10.21
8.	TETRACOSANE	22.93	3.37
9.	HEPTADECANOL	23.44	0.69
10.	OCTADECANOL	24.635	2.62

*PERCENTAGE OF VOLATILE CONSTITUENT

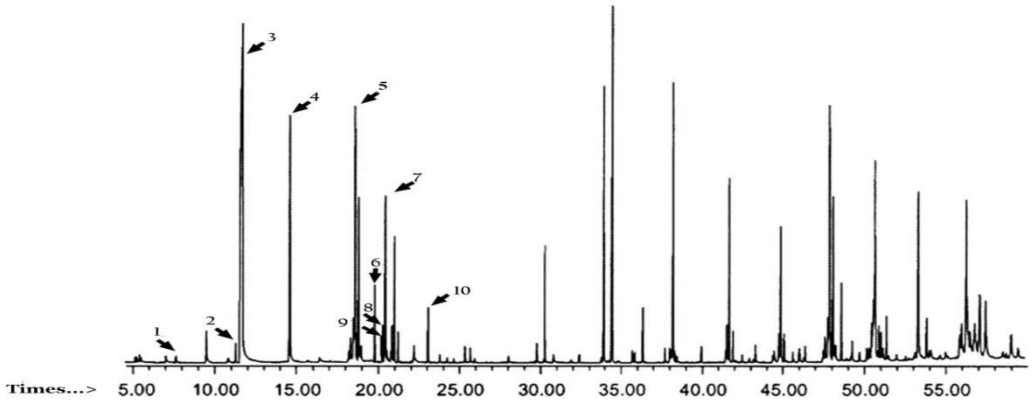

FIG.2: GC-MS ANALYSIS OF SAMPLE-2

TABLE 2: COMPOUND PRESENT IN GC-MS ANALYSIS OF SAMPLE-2

S.NO	COMPOUND	RETENSION TIME(RT)	% of V.C (*)
1.	NONANE	7.600	0.26
2.	DECANE	11.232	0.3
3.	PHENYLETHYLALCOHOL	11.621	2.76
4.	CITRONELLOL	14.562	2.9
5.	PENTADECANE	18.751	7.6
6.	NEROL	19.643	0.50
7.	GERENIOL	20.250	7.2
8.	HEPTADECANOL	20.275	0.54
9.	OCTADECANOL	20.41	1.93
10.	TETRACOSANE	23.089	3.1

*PERCENTAGE OF VOLATILE CONSTITUENT

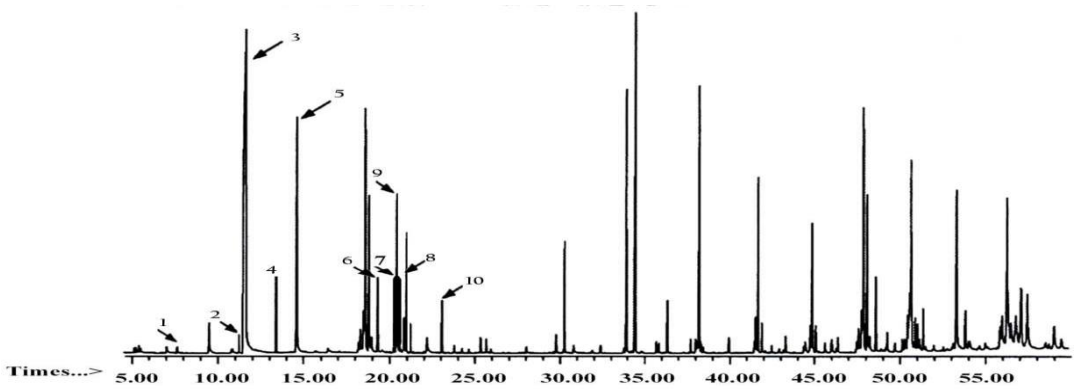

FIG.3: GC-MS ANALYSIS OF SAMPLE-3

TABLE 3: COMPOUND PRESENT IN GC-MS ANALYSIS OF SAMPLE-3

S.NO	COMPOUND	RETENTION TIME	% of V.C (*)
1.	NONANE	7.31	0.2
2.	DECANE	11.20	0.2
3.	PHENYLETHYLALCOHOL	11.43	5.02
4.	PENTADECANE	13.03	6.9
5.	CITRONELLOL	14.62	3.01
6.	NEROL	19.619	0.73
7.	OCTADECANOL	20.12	2.4
8.	GERENIOL	20.191	11.75
9.	HEPTADECANOL	20.21	1.2
10.	TETRACOSANE	23.09	3.32

*PERCENTAGE OF VOLATILE CONSTITUENT

Cytotoxicity,safety Profile

In Acute toxicity studies, all animals in 15 mililiter/70kilogram, 20 mililiterl/70kilogram and 25mililiter/70kilogram adult dose category did not show signs of abnormality throughout 3-days duration of study time, after oral administration of rosewater samples. The single fleeting medical mark that mainly marked at 25 ml/70kg (at very high dose) show mild sedation, animals renowned as tedious and motionless instantly after dosing, but the sign vanished after one hundred and twenty minutes. The motor functions was well with no signs of gait irregularity (deviation from normal walking). Mucous

membranes were healthy in all animals, and there were no visible changes in the colour of eyes. All the animals excluding the control groups defecated semi-formed soggy droppings/pellet that might not fit the account of outright diarrhoea. There was no sign of acute toxicity observe during the forty eight hours study. All group show no death count of animal.. cytotoxicity(brine shrimp lethality assay)outcomes of rosewater samples was refer to table 4,5 and 6 showed that all rosewater samples experienced had LC_{50}> 3000µg/ml, demonstrating that rosewater was almost non-toxic.

TABLE 4: BRINE SHRIMP (*ARTEMIA SALINA*) LETHALITY BIOASSAY OF SAMPLE-1

"Dose µg/ml."	No. of "Shrimps"	No. of "Survivors"	% "Mortality."	"Standard Drug"	% "Mortality."
10	30	26	13.33	Etoposide	46.66%
100	30	24	20		
1000	30	22	26.66		
			Mean ±S.D19.99±6.66		

- Values are recorded as MEAN ± S.D
- LC_{50} =3098.18

TABLE 5:BRINE SHRIMP (*ARTEMIA SALINA*)LETHALITY BIOASSAY OF SAMPLE-2

Dose µg/ml	No. of Shrimps	No. of Survivors	% Mortality	Standard Drug	% Mortality
10	30	25	16.66		
100	30	23	23.33	Etoposide	46.66%
1000	30	18	40		
			Mean ±S.D 26.66±12.02		

- Values are recorded as MEAN ± S.D
- LC_{50} = 1490.95

TABLE 6:BRINE SHRIMP (*ARTEMIA SALINA*)LETHALITY BIOASSAY OF SAMPLE-3

Dose µg/ml."	No. of "Shrimps"	No. of "Survivors"	% "Mortality."	"Standard Drug"	% "Mortality."
10	30	23	23.33	Etoposide	46.66%
100	30	25	16.66		
1000	30	24	20		
			Mean ±S.D19.99±3.33		

- Values are recorded as MEAN ± S.D
- LC_{50} = 3026.0

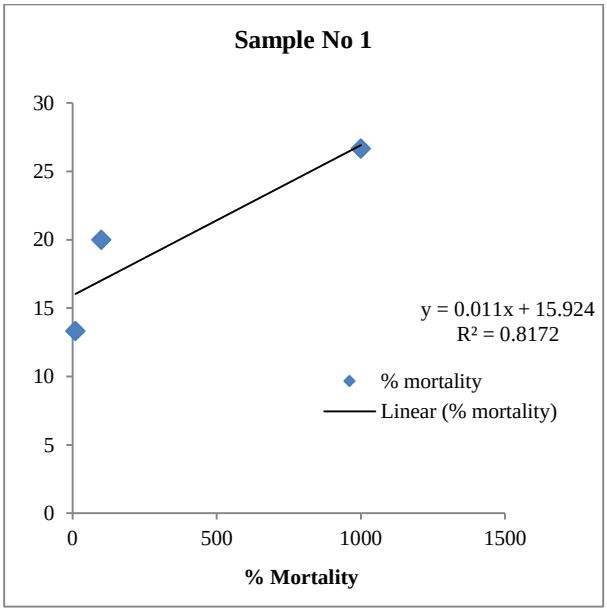

FIG. 4: PERCENT MORTALITY OF SAMPLE 1

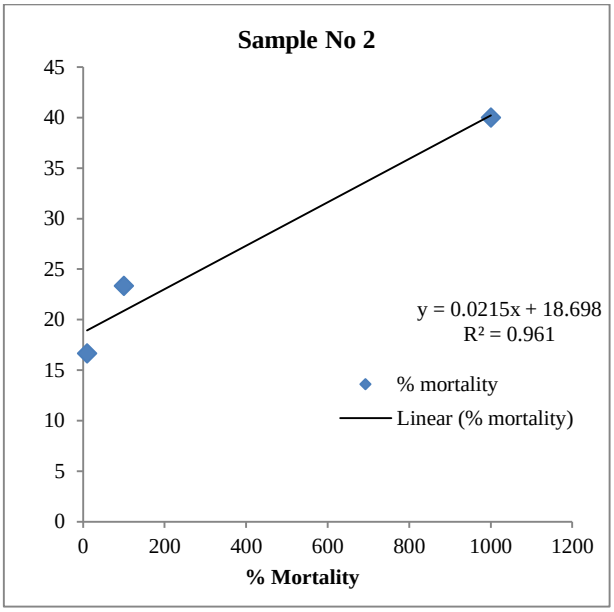

FIG.5: PERCENT MORTALITY OF SAMPLE 2

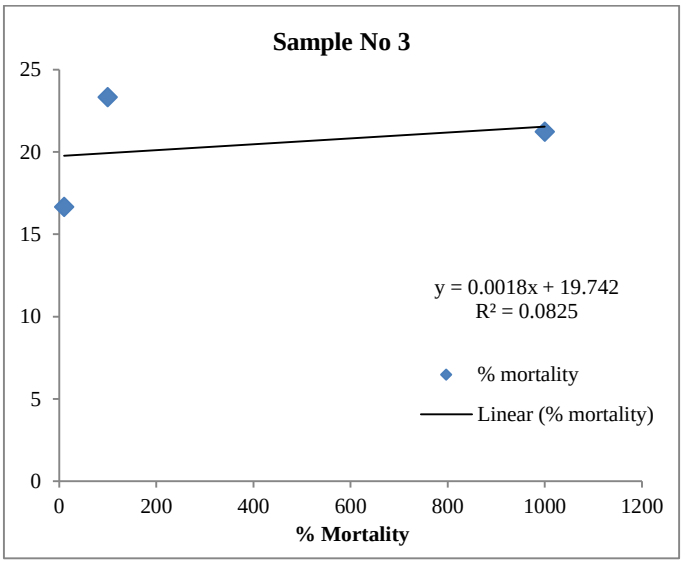

FIG.6: PERCENT MORTALITY OF SAMPLE 3

DISCUSSION:

Rosa damascene, damask rose, damask rose of Castile, gole Mohammadi belongs to the family Rosaceae is one of the vital plants also called as king of flowers. The scent of rose bloom is steady and individual. There are about two hundred varieties of rose existing international. It is supposed that numerous prehistoric plants belong to Rosaceae family (*Rubus idaeus, Prunus armeniaca, Pygenum africanum)* (*Boskabady et al.,2011*). The narration of rose is years aged(about 25-40 million) .Approximately five thousand time reverse Mesopotamian prepared clay tablets of the rose which is the oldest evidence, but many believers say that the Babylonian is the first who use rosewater(*Gudin et al.,2000*) .

Damask rose is used for centuries for their beautifying purpose. Its aromatic fragrance preserved in the form of water extract of rose (rose water) by such long ancient time, and now it is also seen in Indian and Iranian tradition(*Baser et al .,2012*)**.** The rose water samples directly analysed,and it was observed that all samples have all most similar chemical profile qualitatively. Identification and quantification of volatile compounds which were tentatively evaluated from their mass spectra by comparison with library data (Nist), while semi-quantitative values (%) were calculated from peak areas of related components on total area basis without using any response factor. The volatile-compounds recognized in Rose Water samples are listed in Table 1, 2, 3 respectively.

The current learning describes a straight investigation technique (HS–GC–MS) designed for the purpose of volatile compound present in the Rose Water, along with the different operational conditions of headspace, the maximum effectiveness achieved at one hundred and twenty degree celcius °C in 20 min of incubation time. This method recognized approximately fortysix compound for sample#1, thirtyeight compounds for sample# 2 ,3. In evaluation with normally used hydro-distillation (HD), HS–GC–MS technique is establish extremely simple, desires a low quantity of sample, suitable to grip with no by means of any solvent and require shorter examination time. It has elevated trapping capability for

volatile, thermally sensitive compounds. Significant components recognized by this technique were furfuraldehyde derivatives. This isan indication of the presence of higher carbohydrate content. According to the ISO standard(*Verma et al.,2011*), the value of citronellol is about 25.0-46.0% and for phenyl ethyl alcohol greater than 3.5%.

The acute toxicity studies, involve the use of three animals of a single sex per step. The OECD Guideline 423 (2001) provide reproducible technique that uses a small number of animals as per appendices 2, 3 and 4. Rosewater studied for acute toxicity was completed on albino rats (wistar).dose of Rosewater was given orally, examination recorded up to forty eight hours. There was no abnormality seen in the behaviour of animals, no alteration in the respiratory system, the gastrointestinal and execratory system observed, somewhat sedative effect was renowned.

BST method is used to study the cytotoxicity,pesticidal and gastro-protective movement of medicinal plants. This process is safe, reliable, inexpensive and appropriate bioassay tool (Meyer et al., 1982). The results showed that up to 1000µg/ml rosewater samples showed no toxicity. Overall cytotoxicity study highlighted that rosewater as a secure product relating to its convention and appliance.

CONCLUSION:

The present study highlights the simplicity and efficiency of HS–GC–MS technique. It also proves that it is a fast analytical method for direct profiling of volatile compounds in plant-based products containing volatiles compound. 38-46 compounds have been identified in Rose Water samples by using the HS–GC–MS technique. Rose petal is an excellent source of rose water when using water as a solvent. Toxicity studies reflect rose water as a safe and non-toxic drug either used in medicine or cosmetics.

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CONFLICT OF INTEREST:

There is no conflict of interest among all authors in this study.

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